DISSERTATION TITLED

"PREVALENCE OF VITAMIN B12 DEFICIENCY IN TYPE 1 DIABETES MELLITUS AND ITS CAUSES"

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CERTIFICATE

This is to certify that the dissertation **"PREVALENCE OF VITAMIN B12 DEFICIENCY IN TYPE 1 DIABETES MELLITUS AND ITS CAUSES"** is a bonafide work done by **Dr. MIDHUN KUMAR.B** Post Graduate Student, Institute of Internal Medicine, Madras Medical College, Chennai-3, during March 2014 to August 2014 in partial fulfillment of the University Rules and Regulations for the award of MD Branch – I General Medicine, under our guidance and supervision, during the academic year 2012 - 2015.

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DECLARATION

I solemnly declare that the dissertation entitled **"PREVALENCE OF VITAMIN B12 DEFICIENCY IN TYPE 1 DIABETES MELLITUS AND ITS CAUSES"** is done by me at Madras Medical College, Chennai-3 during March 2014 to August 2014 under the Guidance and supervision of **Prof.S.TITO, M.D.,** to be submitted to the Tamilnadu Dr M.G.R Medical University towards the partial fulfillment of requirements for The award of **M.D DEGREE IN GENERAL MEDICINE BRANCH-I.**

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ABBREVATIONS

2-h PG	-	Two hours post prandial plasma glucose
AGE	-	Advanced Glycated End products
ADA	-	American Diabetic Association
ANOVA	-	Analysis of variance
BMI	-	Body mass index
BSA	-	Body surface area
cAMP	-	cyclic adenosine monophosphate
CHD	-	Coronary Heart Disease
CRP	-	C – Reactive Protein
CV	-	Cardio Vascular
DM	-	Diabetes Mellitus
DN	-	Diabetic Nephropathy
DNA	-	Deoxyribonucleic acid
EDTA	-	Ethylene diamine tetra acetic acid

ESRD	-	End Stage Renal Disease
F	-	Adult Females
FPG	-	Fasting Plasma Glucose
GCT	-	Glucose Challenge Test
GDM	-	Gestational Diabetes Mellitus
GFR	-	Glomerular Filtration Rate
GIT	-	Gastro Intestinal Tract
HbA1c	-	GlycatedHaemoglobin.
IFG	-	Impaired Fasting Glucose
IGT	-	Impaired Glucose Tolerance
IF	-	Intrinsic factor
JNK	-	c-Jun N-terminal kinases
LDL	-	Low Density Lipoprotein
MCH	-	Mean corpuscular haemoglobin
MCHC	-	Mean corpuscular haemoglobin concentration

MMA	-	Methyl Malonic Acid
PKA	-	Protein kinase A
RDA	-	Required dietary allowance
RNA	-	Ribo nucleic acid
SAM	-	S-adenosyl- methionine
SDH	-	Succinyl dehydrogenase

CONTENTS

ABSTRACT

BACKGROUND AND PURPOSE:

Vitamin B 12 is an essential micro nutrient, required for optimal hemopoietic, neurologic and cardio vascular function. Auto immune destruction of insulin producing beta cells can produce type 1 diabetes and it's characterized by the presence of insulitis and beta cell auto antibodies. Auto immune gastritis and pernicious anemia are common auto immune diseases present in about 2 % of the population. This prevalence increases to 3 to 5 fold in type 1 diabetes. Presence of parietal cell antibodies and anti-bodies to intrinsic factor has been demonstrated in this population. These factors could contribute to the occurrence of B12 deficiency in these patients.

METHODS:

In our study about 50 patients with type 1 diabetes mellitus were compared with 50 age and sex matched controls and prevalence of vitamin B12 deficiency was assessed. Presence of anti-intrinsic factor antibody was done in those deficient in vitamin B12.

RESULTS:

Our study showed that the prevalence of low serum vitamin B12 in type 1 diabetics when compared with age and sex matched controls. 42 % of type 1 diabetes

patients had low vitamin B12 level using laboratory cut- off value of 180 pmol/L while only 6 % of controls had low vitamin B12 levels(<180 pmol/l). The presence of anti-intrinsic factor antibodies in those deficient in vitamin B12 was done. Among 24 type 1 diabetes mellitus patients who were deficient in vitamin B12 (<180 pmol/l), 4 patients had anti intrinsic factor antibodies. Among 3 individuals in the control group, who were deficient in vitamin B12 (<180 pmol/l), none of them had anti intrinsic factor antibodies, which was statistically significant which stresses the fact that auto immune pathology for vitamin B12 deficiency.

CONCLUSION:

Clinical vitamin B12 deficiency is highly prevalent among patients with type 1 DM. These findings merit further research on a larger population using additional markers to investigate into the cause of deficiency, the factors involved, and benefit of B12 supplementation in these patients. Future large and well-designed studies on screening for vitamin B12 deficiency, vitamin B12 supplementation and optimal supplementation dose among type 1 and type 2 diabetic patients are warranted to help guide formulation of guidelines in diabetes clinical care.

INTRODUCTION

Vitamin B 12 is an essential micro nutrient, required for optimal hemopoietic, neurologic and cardio vascular function[1]. Vitamin B 12 is not synthesized in the humans and should be provided from animal source. The process of absorption of vitamin B12 is a complex process and if disturbed can lead deficiency disease of vitamin B12.Vitamin B 12 deficiency diseases are known to be associated with auto immune disorders.

Auto immune destruction of insulin producing beta cells can produce type 1 diabetes and it's characterized by the presence of insulitis and beta cell auto antibodies. It is associated with other autoimmune endocrine disorders and auto antibodies leading to the development of autoimmune polyglandular syndrome[2].

Auto immune gastritis and pernicious anemia are common auto immune diseases present in about 2 % of the population. This prevalence increases to 3 to 5 fold In type 1 diabetes[3,4,5]. Presence of parietal cell antibodies and anti-bodies to intrinsic factor has been demonstrated in this population[6,7]. These factors could contribute to the occurrence of B12 deficiency in these patients. In addition, the dietary habits which vary from one population to another could also contribute to the deficiency.

This study is done to assess the prevalence of vitamin B 12 deficiency in patients with type 1 diabetes and compare with appropriately age and sex matched controls. We also try to find out pernicious anemia as the cause of vitamin B12 deficiency by testing for the presence of anti-intrinsic factor antibodies.

AIM AND OBJECTIVES

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- To study the “Prevalence of VITAMIN B12 DEFICIENCY in Type 1 Diabetes Mellitus”.

- To correlate the prevalence of vitamin B12 deficiency in Type 1 Diabetes Mellitus with normal population and try to find out pernicious anemia as the cause by testing for anti-intrinsic factor antibodies.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

HISTORY OF DIABETES MELLITUS:

The first historical description of the condition as we know today as Diabetes Mellitus occurred in the Ebers Papyrus, a document of the 18th Egyptian Dynasty of about 1500 BC. In India it was known as “Sweetness of urine” some 1100 years later, and the term 'diabetes' is believed to have been used by the Greeks in the 3rd century BC[8].The first description is usually credited to Aretaeus of Cappadocia in Asia Minor, in the 1st century AD and was the first physician to describe this condition formally. He largely defined the condition as passing of copious amounts of urine and the loss of body flesh[8,9].

Although diabetes was recognized in various writings (including from China), it was Willis in 1674 who re described “sweetness of the urine” and made the deduction that this must be secondary to sweetness of the blood[8,9]. Nevertheless, it was another 100 years later it was demonstrated that this sweetness was due to excess sugar, and a further 50 years later this was recognized as glucose[8.]

It was in the 19th century which saw the major advances in the description of diabetes, its dietary management, its biochemistry and the role of pancreas in the pathology of diabetes. Indeed a link between chronic pancreatitis and diabetes had already been noted at the end of the 18th

century but the missing pancreatic factor was made only after the descriptions of glycosuria following pancreatectomy in dogs by von Mering and Minkowski in 1890.The 19th century also saw the recognition that diabetes might not be a single disease, with a different natural history when presenting in childhood compared to late adulthood. The first clinical descriptions of coma in association with acidosis were also made at this time, and Bernard set the biochemical basis of the condition with his observations on the production of glucose by the liver [8.]

Although descriptions of the treatment of diabetes appear in some of the very early documents, it is often difficult to appreciate whether the diets and herbal remedies offered represent specific therapies or whether they were merely parts of the regular therapeutic armory available for serious disease at that time. Again it was nearly the 19th century before dietary management was formally introduced by Rollo, and throughout that Century a host of dietary measures were suggested for the condition[8.]

Towards the end of 19th century the history of diabetes is dominated by the observations leading to the isolation of insulin, following the discoveries of Minkowski, and the demonstration by Opie of degeneration of the islets of Langerhans. Numerous attempts at insulin extraction were recorded after 1890, and some of the preparations (for example by De Witt, Zuelzer, Paulesco) appear to have had glucose lowering activity. While it

seems likely that a therapeutic preparation would have been made in any case within a couple of years, it fell to the drive and ideas of Banting, the technical support of Best, the facilities and guidance of MacLeod and the biochemical expertise of Collip to produce an adequately pure and potent preparation of bovine insulin in 1922[8.]

Following decades were most clearly marked by the development of the extended acting insulin preparations by Hagedorn and Hallas-Moller, and the accidental discoveries of the hypoglycaemic effects of the biguanide and sulphonylurea groups of drugs. It is yet unclear whether the introduction of highly purified insulin preparations, glycosylated haemoglobin estimation, self-blood glucose monitoring, islet and pancreas transplantation, insulin infusion pumps and genetically engineered insulin will lead to history judging the period between 1973 and 1983 as the most significant in the understanding and management of diabetes[8.]

TECHNICAL MILESTONES IN THE MANAGEMENT OF DIABETES[8.]

Year	Worker(s)	Milestone
1797	Rollo	Dietary management
1913	Alien	Severe calorie restriction
1921	Banting, Best, Collip, Macleod	Isolation and use of insulin
1936	Hagedorn and colleagues	Protamine-insulin complexes used
1939	Loubatieres	Discovery of Sulphonylureas
1970	Jorgensen and Colleagues	Highly purified insulin available
1976	Sonksen and colleagues Walford and Colleagues	Self blood glucose monitoring
1976	Koenig and colleagues	Glycosylated haemoglobin to Monitor control
1979	Goeddel and colleagues	Genetically engineered insulin
1980	Many researchers	Microcomputers in diabetes management

CLASSIFICATION OF DIABETES MELLITUS;

Diabetes is classified into four main groups based on known pathological and etiologic mechanisms-type 1, type 2, other specific types, and Gestational diabetes. Type 1 diabetes results from pancreatic islet cell destruction most commonly by an autoimmune process. These patients are prone to developing ketoacidosis and require insulin replacement. Type 2 diabetes the most prevalent form of diabetes, is a heterogeneous disorder most commonly associated with insulin resistance with or without abnormalities of insulin secretion.

TABLE 1: CLASSIFICATION OF DIABETES MELLITUS[12.]

ETIOLOGICAL CLASSIFICATION	CHARACTERSTICS
Type 1 A	Immune mediated
Type 1 B	Immune destructive, not auto immune
Type 2	Insulin resistance +/- insulin secretory defects
Other specific types	Mitochondrial, maturity onset diabetes of young, endocrinopathies, lipo dystrophy, drug induced, infections
Gestational diabetes	

TABLE 2:

DIFFERENCES BETWEEN TYPE 1 AND TYPE 2

DIABETES MELLITUS

	Type 1	Type 2
Previous terminology	Insulin-dependent diabetes mellitus, type I, juvenile-onset diabetes	Non–insulin-dependent diabetes mellitus, type II, adult-onset diabetes
Age of onset	Usually <30 yr, particularly childhood and adolescence, but any age	Usually >40 yr, but increasingly at younger ages
Genetic predisposition	Moderate; environmental factors required for expression; 35%-50% concordance in monozygotic twins; several candidate genes proposed	Strong; 60%-90% concordance in monozygotic twins; many candidate genes proposed; some genes identified in maturity-onset diabetes of the young
Human leukocyte antigen associations	Linkage to DQA and DQB, influenced by DRB3 and DRB4 (DR2 protective)	None known
Other associations	Autoimmune; Graves disease, Hashimoto thyroiditis, vitiligo, Addison disease, pernicious anemia	Heterogeneous group, ongoing subclassification based on identification of specific pathogenic processes and genetic defects
Precipitating and risk factors	Largely unknown; microbial, chemical, dietary, other	Age, obesity (central), sedentary lifestyle, previous gestational diabetes (see Table 69-1)
Findings at diagnosis	85%-90% of patients have one and usually more autoantibodies to ICA512, IA-2, IA-2β, GAD_{65}, IAA	Possibly complications (microvascular and macrovascular) caused by significant hyperglycemia in the preceding asymptomatic period
Endogenous insulin levels	Low or absent	Usually present (relative deficiency), early hyperinsulinemia
Insulin resistance	Only with hyperglycemia	Mostly present
Prolonged fast	Hyperglycemia, ketoacidosis	Euglycemia
Stress, withdrawal of insulin	Ketoacidosis	Nonketotic hyperglycemia, occasionally ketoacidosis

TYPE 1 DIABETES MELLITUS;

Understanding the pathogenesis of type 1 diabetes mellitus (type 1 DM) is crucial for developing preventive strategies. It is estimated that 50% to 90% of type 1 DM patients have evidence of auto-antibodies and they are labelled as type 1A DM or autoimmune type 1 DM while the remaining are called type 1B DM or idiopathic type 1 DM. The prevalence of type 1B DM is reported to be 5% to 10% in Caucasian populations. Though limited data is available on type 1 DM from India, in one study as much as 45% of recently diagnosed subjects were found to have idiopathic or type 1B DM. Though the exact pathophysiology is unknown for this subtype, various factors like viral infection, toxins, subclinical pancreatitis or some unidentified genetic defects might be responsible[10,11.]

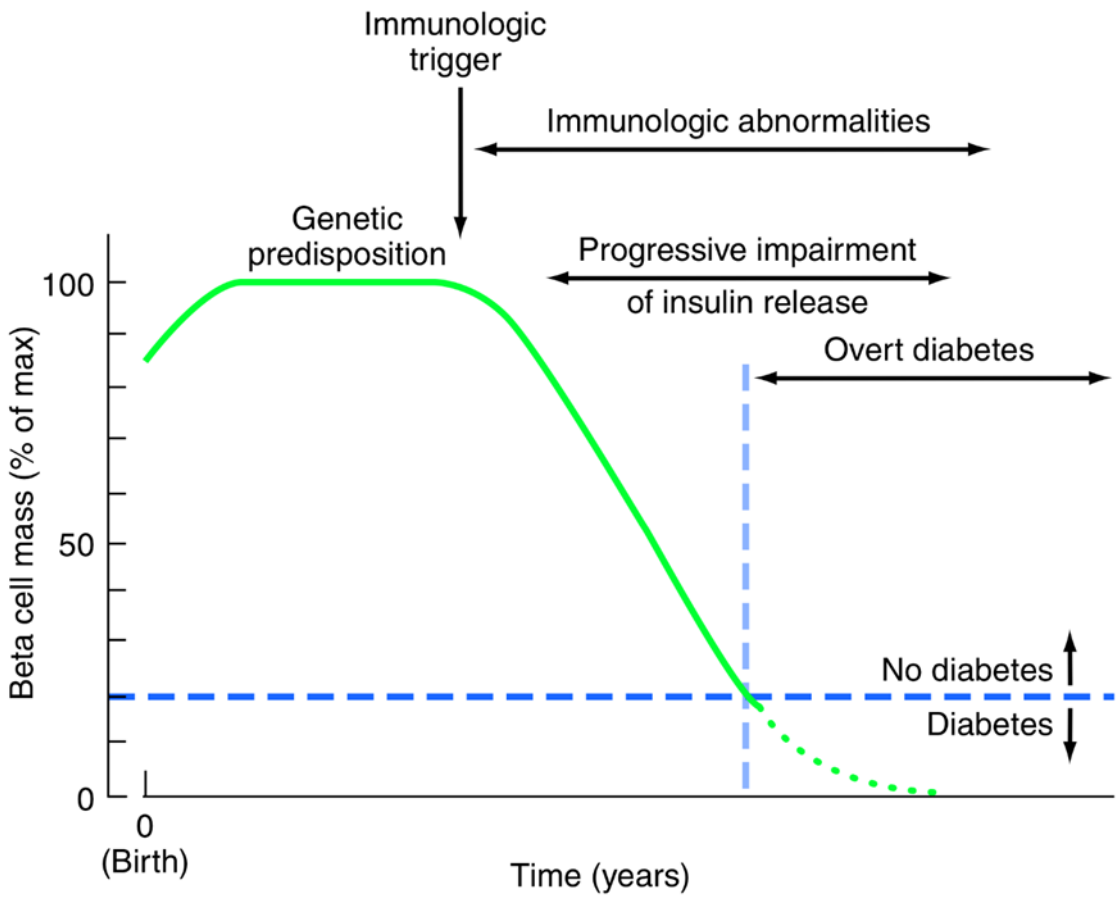


FIGURE 1: TEMPORAL ASSOCIATION OF TYPE 1 DIABETES MELLITUS

In subjects who are genetically predisposed to type 1 DM, certain, as yet, poorly defined environmental triggers initiate T-cell mediated immune response. With the development of insulitis, autoantibodies are produced, which can be detected long before the development of clinical type 1 DM. Insulitis is a slowly progressive disease and over a period of months or years, may cause complete destruction of β-cells (insulinopaenia) leading to clinical manifestation of type 1 DM [10,11].

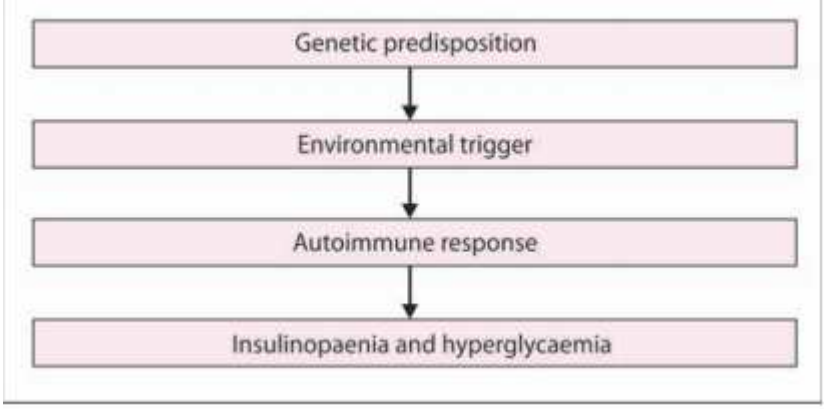


FIGURE 2: PATHOGENESIS OF TYPE 1 DIABETES MELLITUS

Latent autoimmune diabetes of adulthood (LADA) [23]:

Type 1 diabetes can manifest at any age, although most commonly it presents before school age and around puberty. Older adults often present with a more indolent onset that sometimes leads to misdiagnosis and has led to the use of the term latent autoimmune diabetes of adulthood (LADA) to distinguish these patients. These initially unrecognized patients may retain enough cell function at the outset to avoid ketoacidosis, but as their beta cell mass diminishes they develop increasing dependence on insulin therapy. Islet cell antibody studies indicate that up to 15% of patients previously diagnosed with type 2 diabetes may actually have LADA [10,11].

AUTO IMMUNITY AND TYPE 1 DIABETES MELLITUS:

Circulating antibodies against cell proteins, islet cell antibodies (ICA), insulin autoantibodies (IAA), and antibodies to glutamic acid decarboxylase 65 (GAD), tyrosine phosphatase IA2 (ICA512), and zinc transporter 8 (ZnT8) are present in most patients with type 1 diabetes at diagnosis [15,16,18] .These autoreative antibodies can often be detected well before the onset of frank hyperglycemia, even decades earlier, providing evidence that the autoimmune process may be prolonged. After diagnosis, autoantibody levels often decline with increasing duration of the disease. Also, once patients are treated with insulin, low levels of IAA develop, even in patients that do not have an autoimmune etiology for their diabetes [13].

TABLE 3: ANTI BODIES IN TYPE 1 DIABETES MELLITUS[17]

	Sensitivity	Specificity
Glutamic acid decarboxylase (GAD65)	70%-90%	99%
Insulin (IAA)	40%-70%	99%
Tyrosine phosphatase IA2 (ICA512)	50%-70%	99%
Zinc transporter 8 (ZnT8)	50%-70%	99%

Although useful for diagnosing and predicting type 1 diabetes, antibodies against beta cell proteins do not directly cause the destruction of beta cells in type 1 diabetes. Instead, it is the cellular immune system, the T lymphocytes that infiltrate the islets (a process called insulitis) and destroy the beta cells. At the time of diagnosis, the islets of patients with type 1 diabetes are extensively infiltrated with both helper and cytotoxic T lymphocytes[19,20]. Normally, the thymus deletes auto reactive T cells during development so that the immune system becomes tolerant of self–antigens. In addition, certain specialized T cells, the regulatory T cells, further prevent attacks against healthy tissues by retraining the activity of any auto reactive cytotoxic and helper T cells that escape the thymus. Type 1 diabetes results from a breakdown in these processes of self-tolerance in the immune system[16,17].

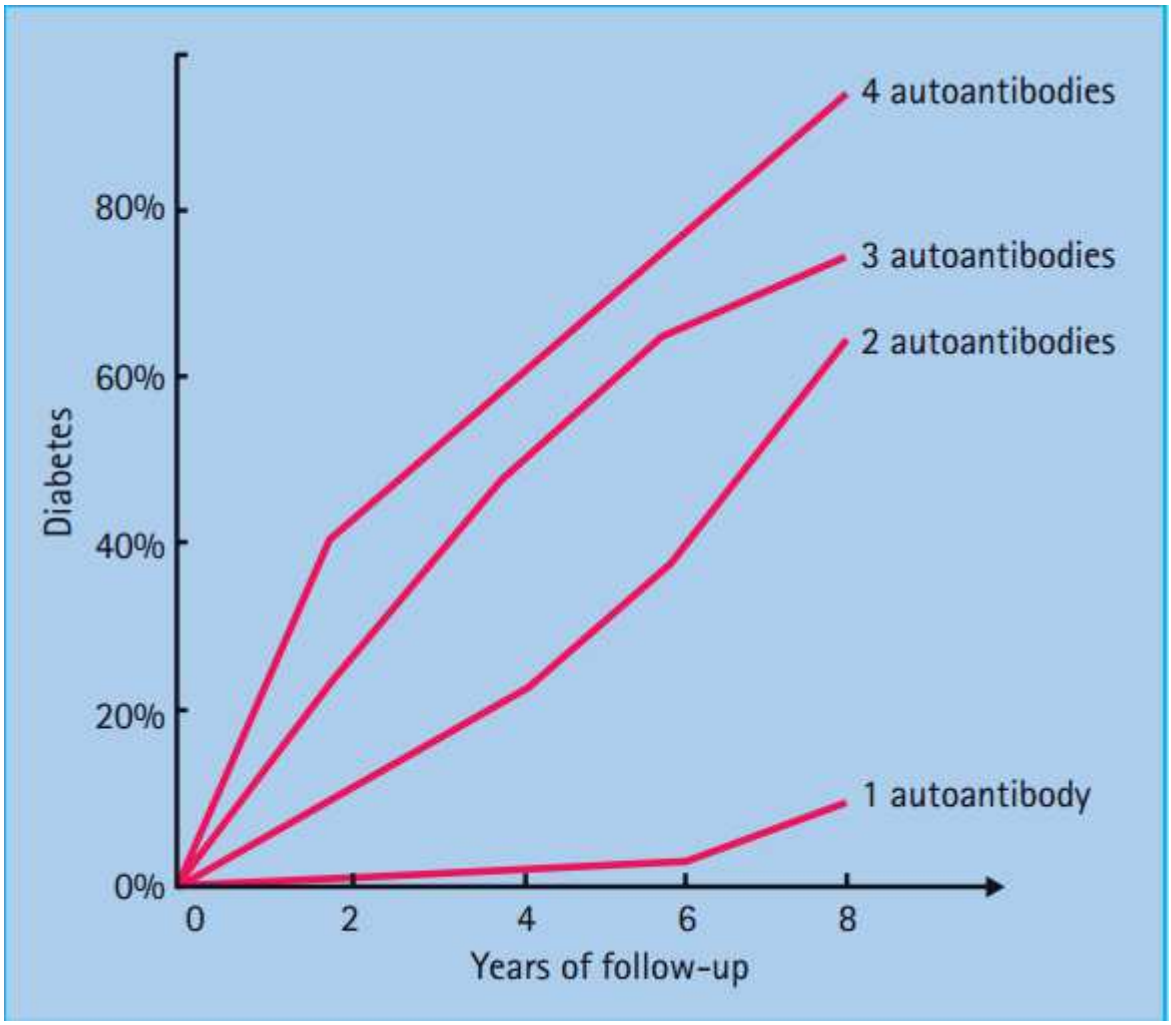


FIGURE 3:EFFECTS OF MULTIPLE ISLET AUTOANTIBODIES ON THE RISK OF TYPE 1 DIABETES (T1DM)

PATHOGENESIS OF TYPE 1 DIABETES MELLITUS:

Type 1 diabetes constitutes less than 2% of total diabetic population in India. In majority of type 1 diabetes patients, there is rapid destruction of β-cells of pancreas, in a susceptible subject due to viral mediated autoimmune process. A typical type 1 diabetes patient is below 30 years, is underweight and present with frank symptoms, e.g. polyuria, polydipsia, polyphagia, weakness, weight loss, restlessness and if continued for some period, may lead to diabetic ketoacidosis with altered sensorium and severe dehydration. Occasionally a child with similar clinical presentation in a remote area may die before the diagnosis is made. Any comatose child presenting with severe dehydration without diarrhea, a diagnosis of type 1 diabetes should be in the list of diagnostic consideration. In some type 1 diabetic subjects, β-cell destruction is slower and may mimic a type 2 diabetes in clinical presentation (latent autoimmune diabetes in adult (LADA)[23]. After initial treatment with insulin, a type 1 diabetic may recover some residual β-cell function (the so-called 'honeymoon phase') when they can be maintained with a small daily dose of insulin; rarely, they do not even need insulin for some period of time. However, this phase of recovery of residual β-cell function is temporary and the autoimmune process ultimately destroys the remaining β-cells and the subject becomes completely insulin deficient and require insulin for survival [13].

GENETICS OF TYPE 1 DIABETES MELLITUS;

Increased lifetime risk of developing type 1 diabetes is present in Family members of patients with type 1 diabetes. The offspring of a mother with type 1 diabetes have a risk of 3%, whereas the risk is 6% for children of affected fathers. The risk in siblings of affected individuals is related to the number of human leukocyte antigen (HLA) haplotypes that the sibling shares [32-34]. If one haplotype is shared, the risk is 6% and if two haplotypes are shared, the risk increases to 12% to 25%. For monozygotic twins, the concordance rate reaches 25% to 50%. Although these data demonstrate a strong genetic contribution to the risk of type 1 diabetes, genetics plays an even larger role in type 2 diabetes, and environment also clearly contributes substantially to the risk of type 1 diabetes[16,17].

Genes in the major histocompatibility (MHC) locus on the short arm of chromosome 6 explain at least half of the familial aggregation of type 1 diabetes. Within the MHC locus lie a number of closely packed genes involved in the function and regulation of the immune response. Although a number of genes within the MHC locus have been linked to the risk of developing type 1 diabetes, the most important of these are the genes encoding the HLA class II molecules DQ and DR. The professional antigen-presenting cells, dendritic cells, macrophages and B lymphocytes, use the

class II molecules on their cell surface to present peptide antigens to T lymphocytes through the T-cell receptor. T cells activated by antigen-presenting cells carry out the cell destruction that leads to type 1 diabetes. Although exact mechanisms remain uncertain, the variations in the amino acid sequence of individual HLA class II molecules may impact their ability to present specific self-peptides to T cells either in the process of central or peripheral tolerization or later during the development of the autoimmune response, thereby contributing to the risk of developing type 1 diabetes[16,17.]

The DR Haplotypes DR3 and DR4 are major susceptibility risk factors for type 1 diabetes. As many as 95% of type 1 diabetic patients have a DR3 or a DR4 haplotype—or both—compared with 45% to 50% of Caucasian non diabetic controls. Individuals who express both a DR3 and a DR4 allele carry the highest risk for type 1 diabetes [24,25].

An independent genetic link to chromosome 11 has also been identified in type 1 diabetes. Studies of a polymorphic DNA locus flanking the 5' region of the insulin gene on chromosome 11 revealed a small but statistically significant linkage between type 1 diabetes and this genetic locus. This polymorphic locus, which consists of a variable number of tandem repeats (VNTRs) with two common sizes in Caucasians, small (26-63 repeats) or large (140-243 repeats), does not encode a protein[27-30]. An intriguing proposal to explain how the VNTR might influence susceptibility

to type 1 diabetes was based on findings that insulin gene transcription is facilitated in the fetal thymus gland by the presence of the large allele of the VNTR locus flanking the insulin gene. The large VNTR allele might produce a dominant protective effect by promoting negative selection (deletion) by the thymus of insulin-specific T lymphocytes that play a critical role in the immune destruction of pancreatic cells.

The established genetic association with the MHC region of chromosome 6 contributes much more (about 50%) to the genetic susceptibility to type 1 diabetes than does this locus flanking the insulin gene on chromosome 11, which contributes about 10%. Both candidate gene studies and genome-wide association studies (GWAS) have identified a number of additional risk loci that make smaller contributions to the genetic risk of type 1 diabetes. Many of the genes linked to these additional loci also play important roles in the function and regulation of the immune response[27-31].

Mutations in two genes involved in T-cell tolerance cause rare syndromes of type 1 diabetes together with other autoimmune diseases. In the autosomal recessive disease autoimmune polyglandular syndrome type 1, homozygous mutations in the gene encoding the autoimmune regulator (AIRE) prevent the expression of certain self-proteins in the thymus, thus allowing mature auto reactive T cells to leave the thymus. In addition to other

autoimmune diseases and mucocutaneous candidiasis, approximately 20% of patients with APS1 develop type 1 diabetes[32-34.] The second gene, FOXP3, found on the X chromosome, encodes a transcription factor required for the formation of regulatory T cells. Mutations in FOXP3cause immunodysregulationpolyendocrinopathyenteropathy X-linked (IPEX) syndrome. IPEX presents in male patients with very early onset type 1 diabetes, often neonatal, combined with other autoimmune endocrinopathies, autoimmune skin disorders, diarrhea secondary to autoimmune enteropathy, and frequent severe infections [14.]

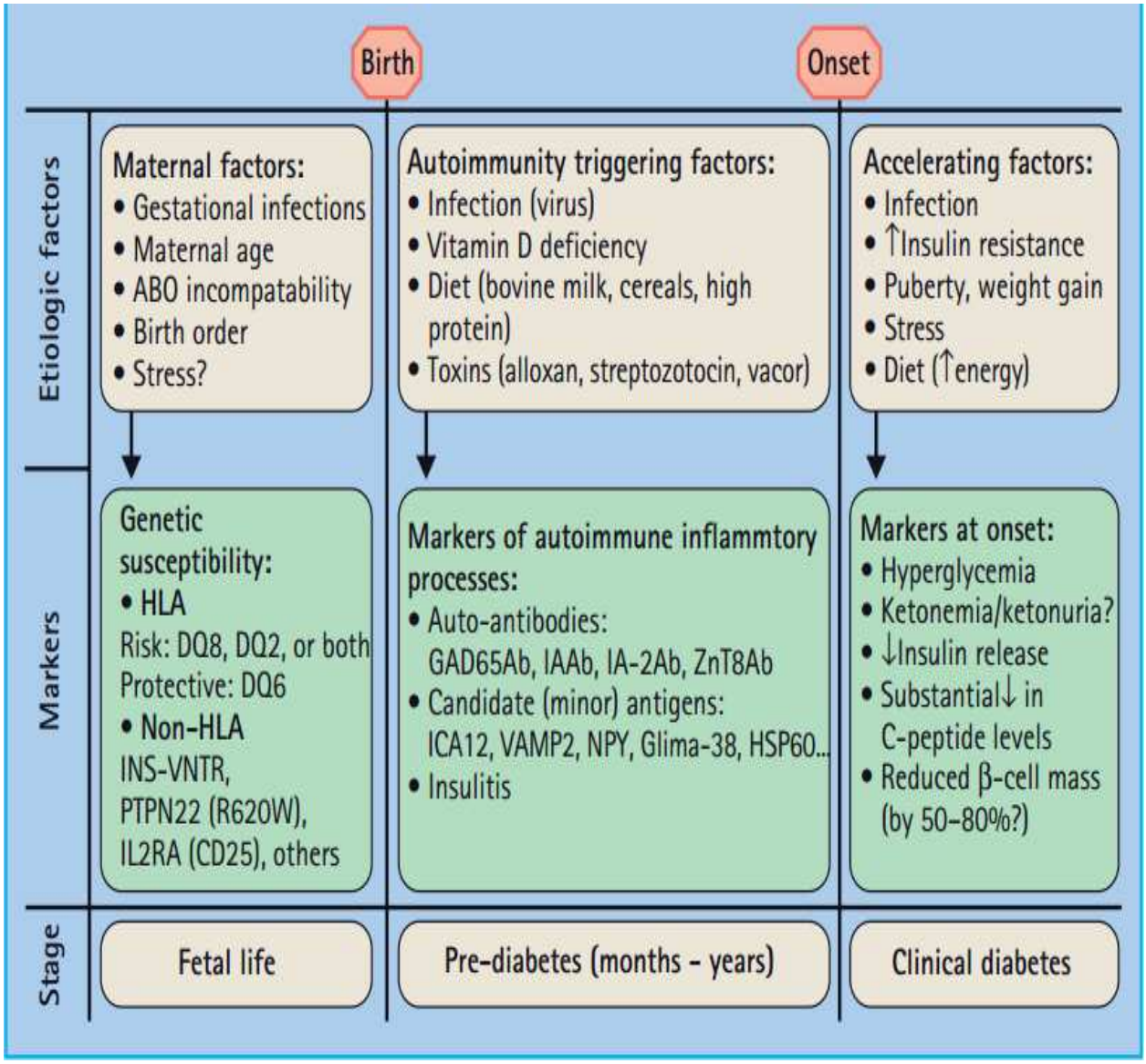


FIGURE 4: NATURAL HISTORY OF TYPE 1 DIABETES.

ENVIRONMENTAL FACTORS IN TYPE 1 DIABETES:

While genetic inheritance may play an important role in causing type 1 diabetes, the monozygotic twin studies demonstrate that other causes,stochastic or environmental, are at least as important. Most individuals with type 1 diabetes do not have other family members with the disease. vacor (a nitrophenylurea rat poison), spoiled tapioca orcassava root and viruses (mumps, congenital rubella, Coxsackie virus B4) have increased risk of developing diabetes How these environmental insults lead to type 1 diabetes is unknown; they may directly damage cells in some cases, or may actas initiators or accelerators of the autoimmune attack on the cells [35-41]. In some cases, molecular mimicry, wherein the immune system mistakenlytargets cell proteins that share homologies with certain viral or other foreign peptides may play a role.

Epidemiological studies have demonstrated an association between breast-feeding in the first 6 months of life and protection from type 1 diabetes. While it has been suggested that proteins in cow's milk may be the culprits, the strongest evidence supports the idea that human breast milk may reduce the risk of autoimmune disease.

TABLE 4 :

ENVIRONMENTAL RISK FACTORS ASSOCIATED WITH TYPE 1 DIABETES MELLITUS (T 1 DM).

Factor	Proposed effect mechanisms	Examples
Maternal factors	Triggering autoimmune response	Gestational infections
	Unknown	Higher maternal age
	Unknown	Higher birth order
	Unknown	ABO blood group incompatibility
Virus infections	Direct β-cell killing (cytolysis)	Mumps virus
	Mimicry of β-cell autoantigens	Rubella virus
	Autoreactive T-cell activation and subsequent β-cell killing	Enterovirus/Coxsackie B virus
		Rotavirus
	Inhibition of insulin production through inducing expression of HLA genes and interferon	Cytomegalovirus
		Epstein–Barr virus
Dietary factors	Triggering autoimmune response	Bovine milk/short breastfeeding
	Triggering autoimmune response	Cereals
	Unknown	High protein content
	Lack of possible protective effect of vitamin D	Vitamin D deficiency
Factors related to insulin sensitivity and/or resistance	Stressing β-cells with excess demands "accelerator hypothesis"	Puberty
		High energy food
	Increase insulin resistance	Weight gain
Psychologic stress	Affect hypothalamic-pituitary-adrenal axis leading to disturbance in autonomic nervous system and autoimmune dysregulation	Stress during pregnancy
		Child–parent separation
		Behavioral deviances
		Difficult adaptation
Toxic substances	Direct damage to β-cells	Alloxan

Accumulating evidence shows that in the process of modernizing and improving public health, the risk of type 1 diabetes has increased, possibly due to the removal of some protective factors. Type 1 diabetes is almost unheard of in many third-world countries, and has its highest incidence in countries with the best public health systems, such as the Scandinavian countries. In addition, the incidence of the disease has been steadily increasing over the past century in western and westernizing countries and is especially high among the more affluent. This has led to the suggestion that a dirty environment, one with more infections (especially more parasitic diseases) and more antigen exposure, may reduce the risk of type 1 disease[35,41].

CLINICAL FEATURE OF TYPE 1 DIABETES MELLITUS:

Patients with type 1 diabetes present with symptoms and signs related to hyperglycemia and hyperketonemia. The severity of the insulin deficiency and the acuteness with which the catabolic state develops determine the intensity of the osmotic and ketotic excess.

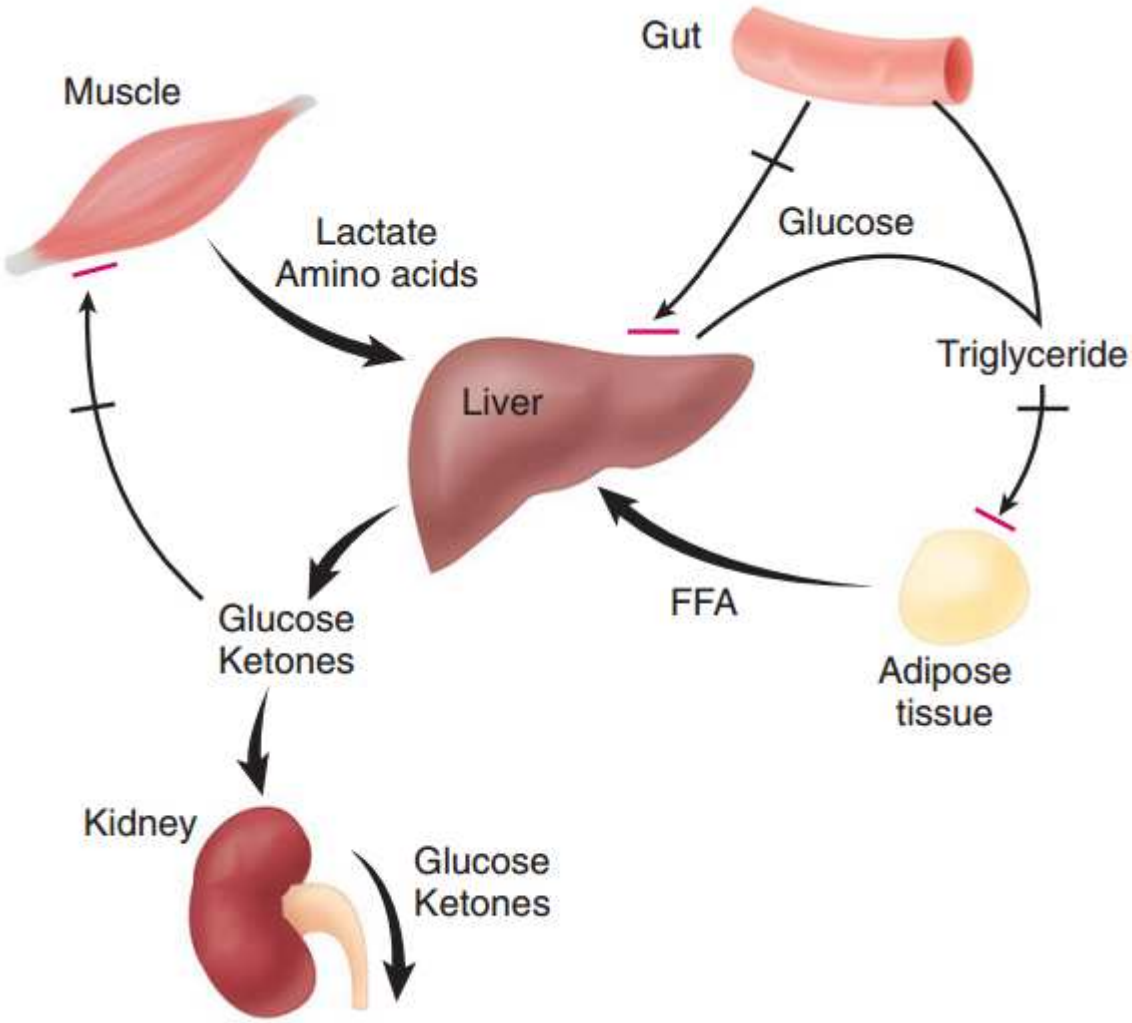


FIGURE 5:

EFFECT OF INSULIN DEFICIENCY ON

BODY FUEL METABOLISM

DIAGNOSIS OF DIABETES:

TABLE 5: DIAGNOSIS OF DIABETES MELLITUS[12]

Measure	American Diabetes Association	
	Diabetes	Prediabetes
Fasting plasma glucose	≥126 mg/dl	100–125 mg/dl (IFG)
2-Hr plasma glucose (during an OGTT with a loading dose of 75 g)	≥200 mg/dl	140–199 mg/dl (IGT)
Casual (or random) plasma glucose (in a patient with classic hyperglycemic symptoms)	≥200 mg/dl	
Glycated hemoglobin	≥6.5%	5.7–6.4%

STAGES OF DIABETES

Stages of diabetes range from normal glucose tolerance through impaired glucose tolerance and impaired fasting glucose, into frank diabetes mellitus, which may be non-insulin requiring, insulin requiring for survival. Type 1 DM can be found across the whole spectrum. In the early stages of treatment there can be a period of non- insulin requirement, but later followed by insulin requirement for survival. In type 2 DM, insulin may be required during a period of ketoacidosis precipitated by severe stress or infection.

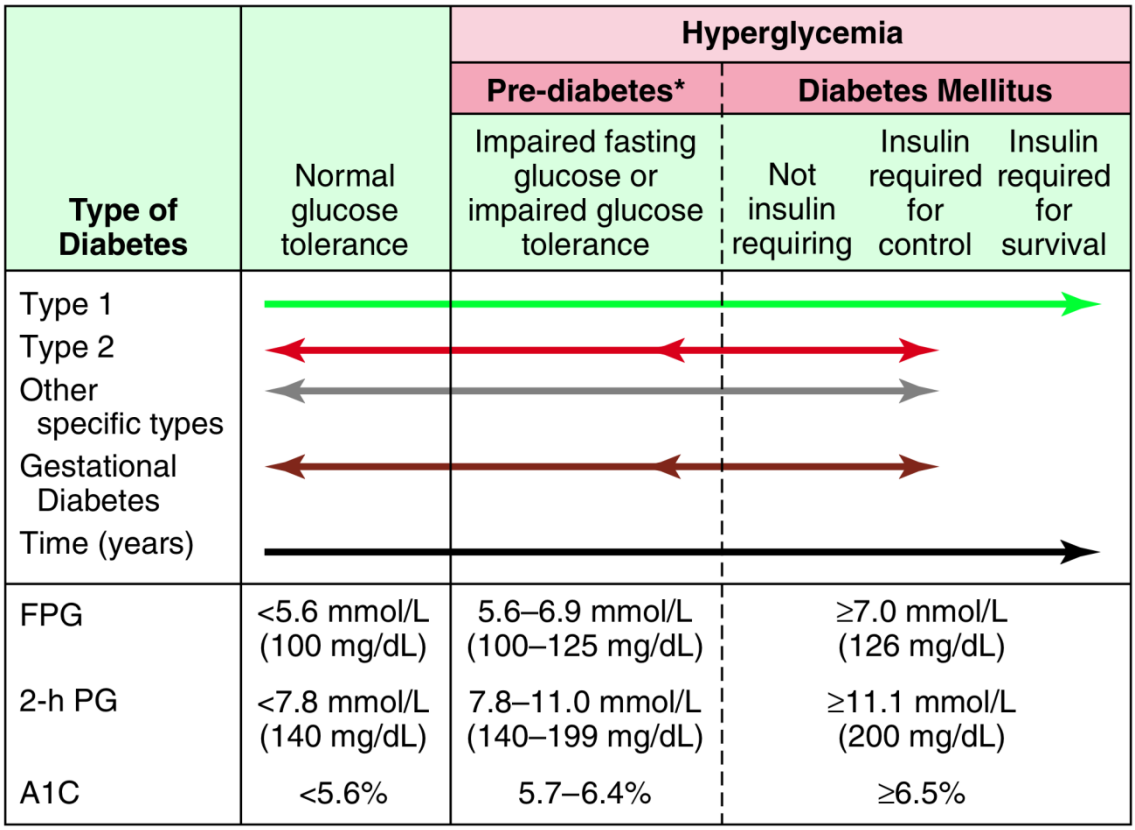


FIGURE 6:

CLASSIFICATION OF DIABETES MELLITUS

COMPLICATIONS OF DIABETES[59-61]:

Eyes

- Diabetic retinopathy
    - Nonproliferative (background)
    - Proliferative
- Cataracts
    - Subcapsular (snowflake)
- Nuclear (senile)

Kidneys

- Intercapillaryglomerulosclerosis
    - Diffuse
    - Nodular
- Infection
    - Pyelonephritis
    - Perinephric abscess
    - Renal papillary necrosis
- Renal tubular necrosis
    - Following dye studies (urograms, arteriograms)

Nervous System

- Peripheral neuropathy
    - Distal, symmetric sensory loss
- Motor neuropathy
    - Foot drop, wrist drop
    - Mononeuropathy multiplex (diabetic amyotrophy)
    - Cranial neuropathy
    - Cranial nerves III, IV, VI, VII
- Autonomic neuropathy
    - Postural hypotension
    - Resting tachycardia
    - Loss of sweating
    - Gastrointestinal neuropathy
    - Gastroparesis
    - Diabetic diarrhea
    - Urinary bladder atony
    - Impotence (may also be secondary to pelvic vascular disease).

Skin

- Diabetic dermopathy (shin spots)
- Necrobiosislipoidicadiabeticorum
- Candidiasis
- Foot and leg ulcers
    - Neurotropic
    - Ischemic

Cardiovascular System

- Heart disease
    - Myocardial infarction
    - Cardiomyopathy
- Peripheral vascular disease
    - Ischemic ulcers: gangrene
- Cerebrovascular disease

Bones and Joints

- Diabetic cheiroarthropathy
- Dupuytren contracture
- Charcot joint
- Osteomyelitis

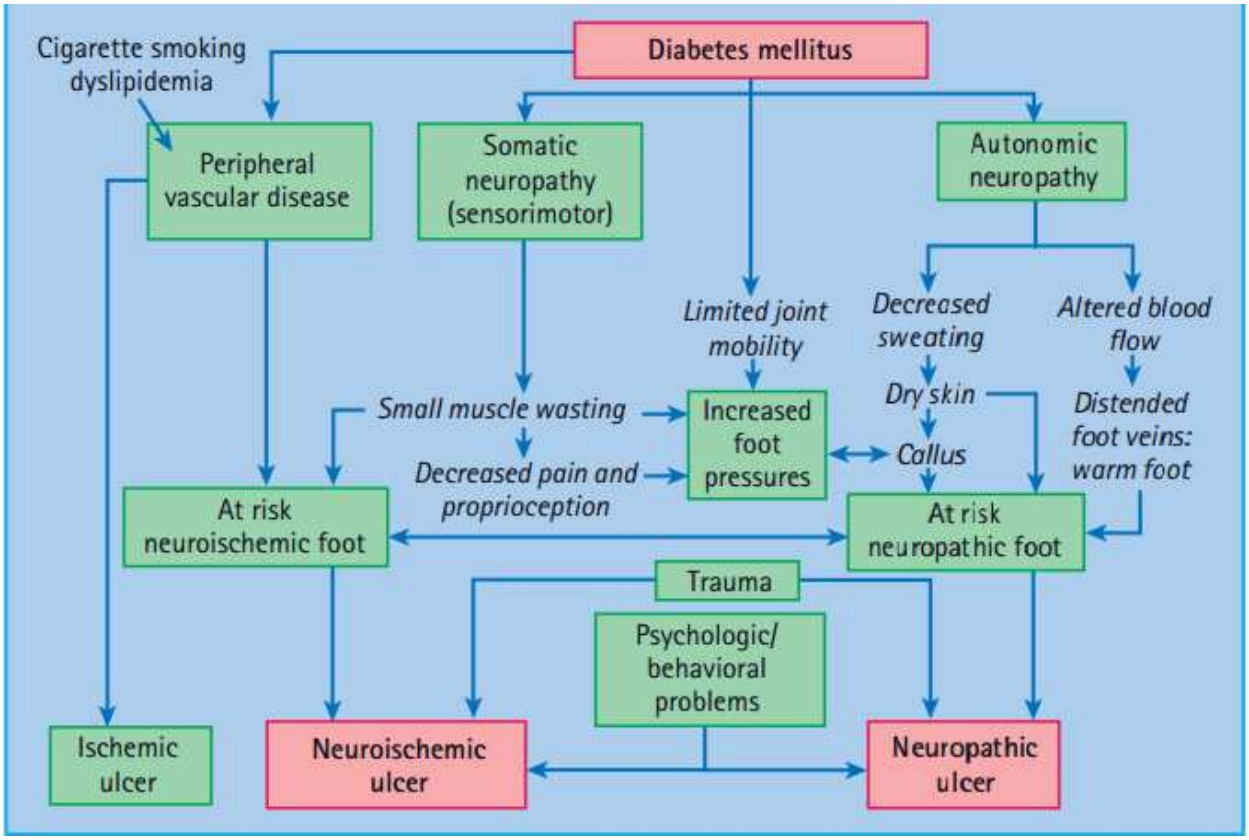


FIGURE 7:

PATHWAYS TO FOOT ULCERATION IN DIABETES

Unusual Infections

- Necrotizing fasciitis
- Necrotizing myositis
- Mucor meningitis
- Emphysematous cholecystitis
- Malignant otitis externa

PREVALENCE OF CHRONIC COMPLICATIONS BY TYPE OF DIABETES:

Although all of the known complications of diabetes can be found in both types of the disease, some are more common in one type than in the other. ESRD occurs more commonly in type 1 diabetic individuals when compared with type 2 diabetes individuals. .proliferative retinopathy causes blindness in type 1, whereas macular edema and ischemia are the usual cause in type 2. Similarly, although diabetic neuropathy is common in both type 1 and type 2 diabetes, severe autonomic neuropathy with gastroparesis, diabetic diarrhea, resting tachycardia, and postural hypotension is much more common in type 1.

PATHOGENESIS OF CHRONIC COMPLICATIONS:

Though hyperglycemia is an important factor, the mechanism of such diverse cellular and organ dysfunction is unknown. Four Prominent theories have been proposed[43-58].

AGEs Theory

Increased intra-cellular glucose causes non enzymatic glycosylation of intra- and extra-cellular proteins by interaction of glucose with amino acid groups on proteins, resulting in formation of advanced glycosylation end products (AGEs). Serum levels of AGEs correlates with the level of glycaemia and these products accumulate as glomerular filtration rate declines. Interaction of AGEs with the receptors in mesangial cells leads to increased transforming growth factor β (TGF-β) expression and extra-cellular matrix synthesis. Aminoguanidine, a compound that inhibits AGEs, was useful in experimental studies but resulted in occurrence of ANCA positive vasculitis and is not approved for clinical use [48,49,50].

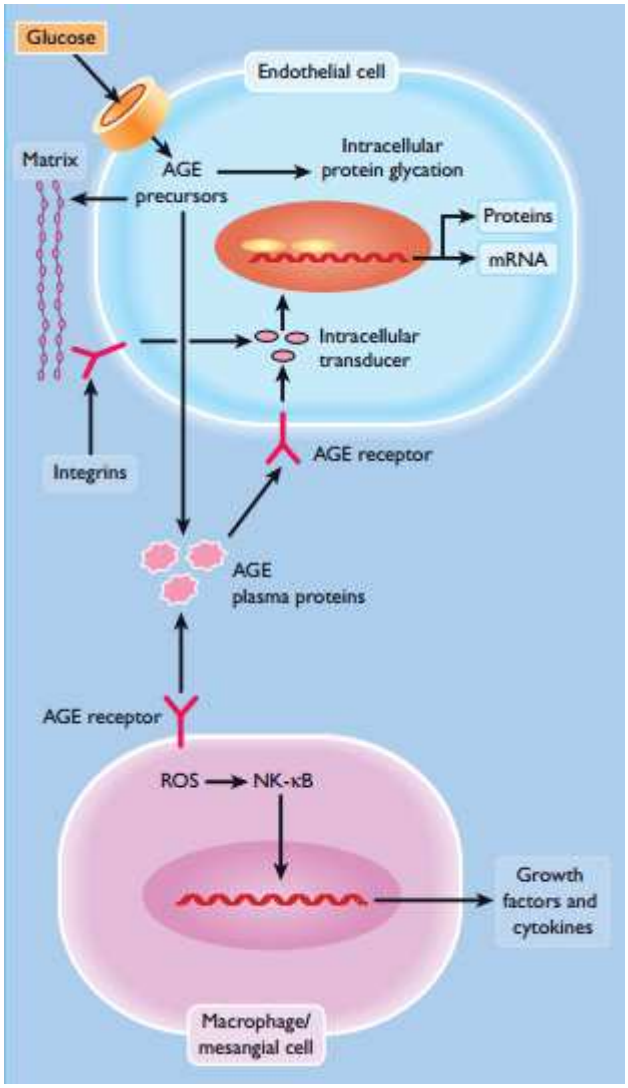


FIGURE 8:

INCREASED PRODUCTION OF INTRACELLULAR ADVANCED GLYCATION END - PRODUCTS (AGE) PRECURSORS

SORBITOL PATHWAY (POLYOL PATHWAY):

Some glucose is converted to sorbitol by the enzyme aldose reductase when the amount of Intra-cellular glucose metabolized by phosphorylation and subsequent glycolysis, is increased. Redox potential is altered by increased sorbitol concentration which alters cellular osmolality, generates reactive oxygen species and increases AGEs formation, and likely and causes cellular dysfunction. Cataract formation and neuropathy and retinopathy to some extent have been linked to this pathway. Newer aldose reductase inhibitors like fidarestat are undergoing clinical trials, epelrestat has been approved for use[51,53].

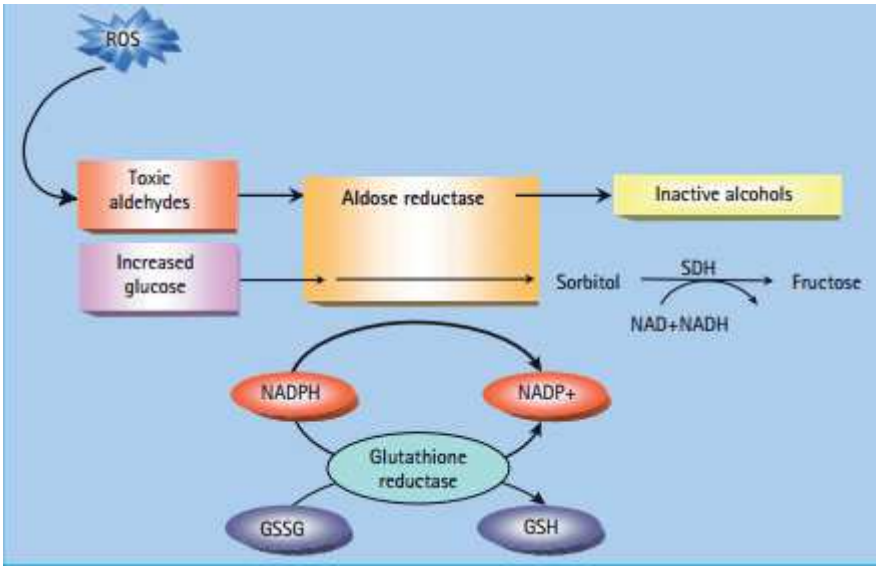


FIGURE 9:

POLYOL PATHWAY

PROTEIN KINASE C PATHWAY:

Hyperglycaemia increases formation of diacylglycerol, leadingto activation of protein kinase C (PKC). PKC is a family of serinethreonine kinases that alter the transcription of genes forfibronectin, type IV collagen, contractile proteins and extracellular matrix protein in endothelial cells and neurons.. PKCmediates TGF-β1, angiotensin-II and vascular endothelialgrowth factor (VEGF) and modulates mitogen activated proteinkinase (MAPK), which mediates sclerosis. Inhibitors of PKC-b likeruboxistaurinmesylate that reduce the direct cellular actionsof AGEs, VEGF, Endothelin-1, reduce oxidised lipids and oxidantproduction, are being studied in clinical trials in diabetesmellitus (DM) for retinopathy and neuropathy[57].

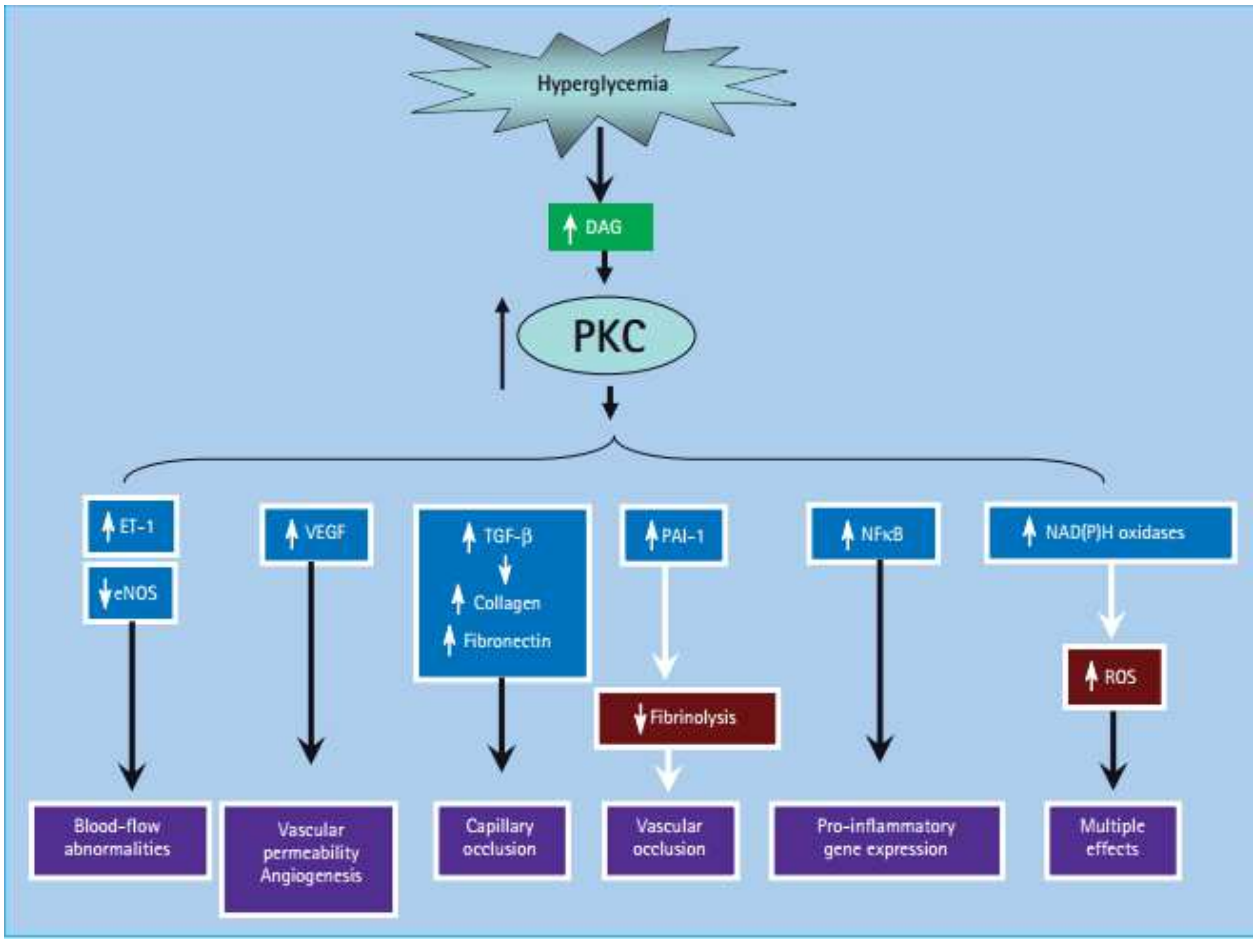


FIGURE 10:

ACTIVATION OF PROTEIN KINASE C (PKC) BY *DE NOVO* SYNTHESIS OF DIACYLGLYCEROL (DAG) AND SOME OF ITS PATHOLOGIC CONSEQUENCES

HEXOSAMINE PATHWAY:

Hexosamine pathway, generates fructose-6-phosphate, which is increased by hyperglycemia. Hexosamine pathway alters the function by causing glycosylation of proteins, such as endothelial nitric oxide synthase, or by changing gene expression of transforming growth factor β (TGF-β) or plasminogen activator inhibitor-1 (PAI-1) [55, 56.]

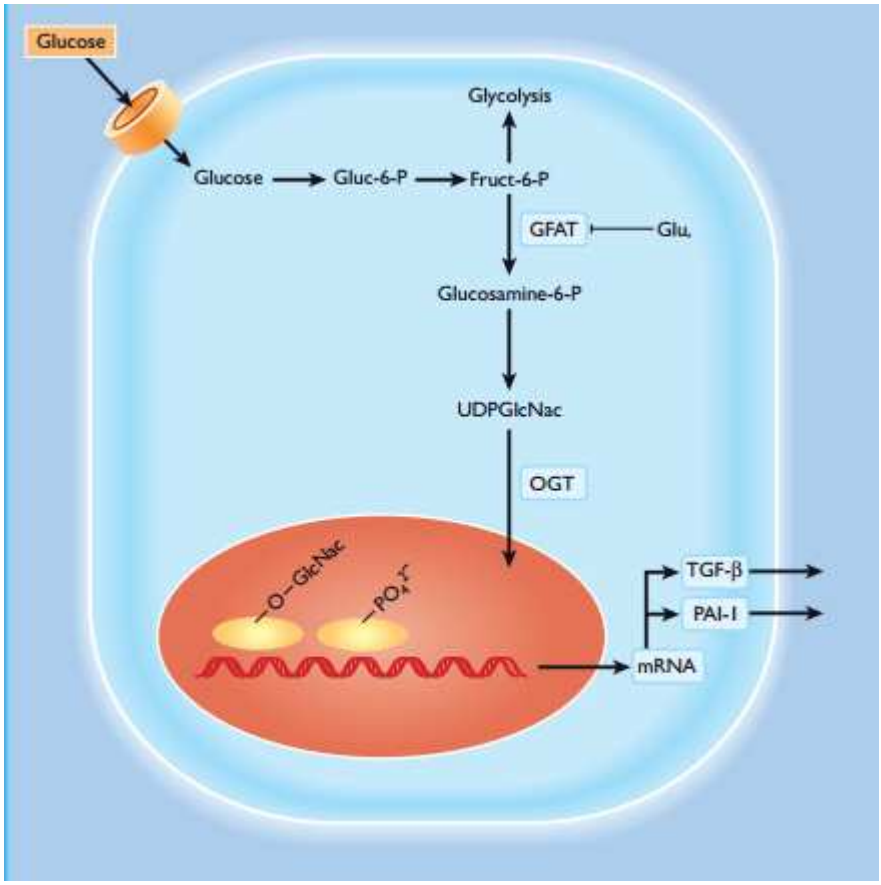


FIGURE 11: HEXOSAMINE PATHWAY

GROWTH FACTORS :

VEGF-A is increased locally indiabetic proliferative retinopathy and decreases after laserphotocoagulation. Inhibition of angiotensin-II also reduces VEGF,which could explain one of the beneficial effects of angiotensinII receptor blockers on microangiopathic diseases. Monoclonalantibodies to VEGF like ranibizumab in experimental studies have shown improvement in proliferative diabetic retinopathy. Other growth factors, such as “platelet derived growth factor, epidermal growth factor, insulin like growth factor, growth hormone, basic fibroblast growth factor, connective tissue growth factor and even insulin”, have been suggested to play a role in DM related complications.

GLYCEMIC CONTROL AND COMPLICATIONS:

Reduction in chronic hyperglycaemia canprevent many early complications of type 1 DM. as suggested by “The diabetes control and complications trial (DCCT) “. This largemulticentre clinical trial randomized 1,400 type 1 diabetics toeither intensive or conventional diabetes management andprospectively evaluated the development of retinopathy,nephropathy, and neuropathy. The intensive group achieved asubstantially lower HbA1c (7.3%) than the conventionallymanaged group (9.1%).DCCT showed that improvement of glycaemic control reducednon-

proliferative and proliferative retinopathy by 47%,microalbuminuria by 39%, and clinical nephropathy by 54% andneuropathy by 60%. Improved glycaemic control also slowedthe progression of early diabetic complications. A nonsignificant trend in reduction of macro vascular events wasobserved during the trial (most individuals were young, with allow risk of cardiovascular disease). Results of DCCT predictedthat type-1 diabetics with intensive management gain 7.7additional years of vision, 5.8 additional years free from ESRDand 5.6 additional years free from lower limb amputations. If allthe complications were combined, intensively managed group would experience 15.3 more years of life without significant micro vascular or neurological complications of diabetes as compared to diabetics receiving standard therapy [63].

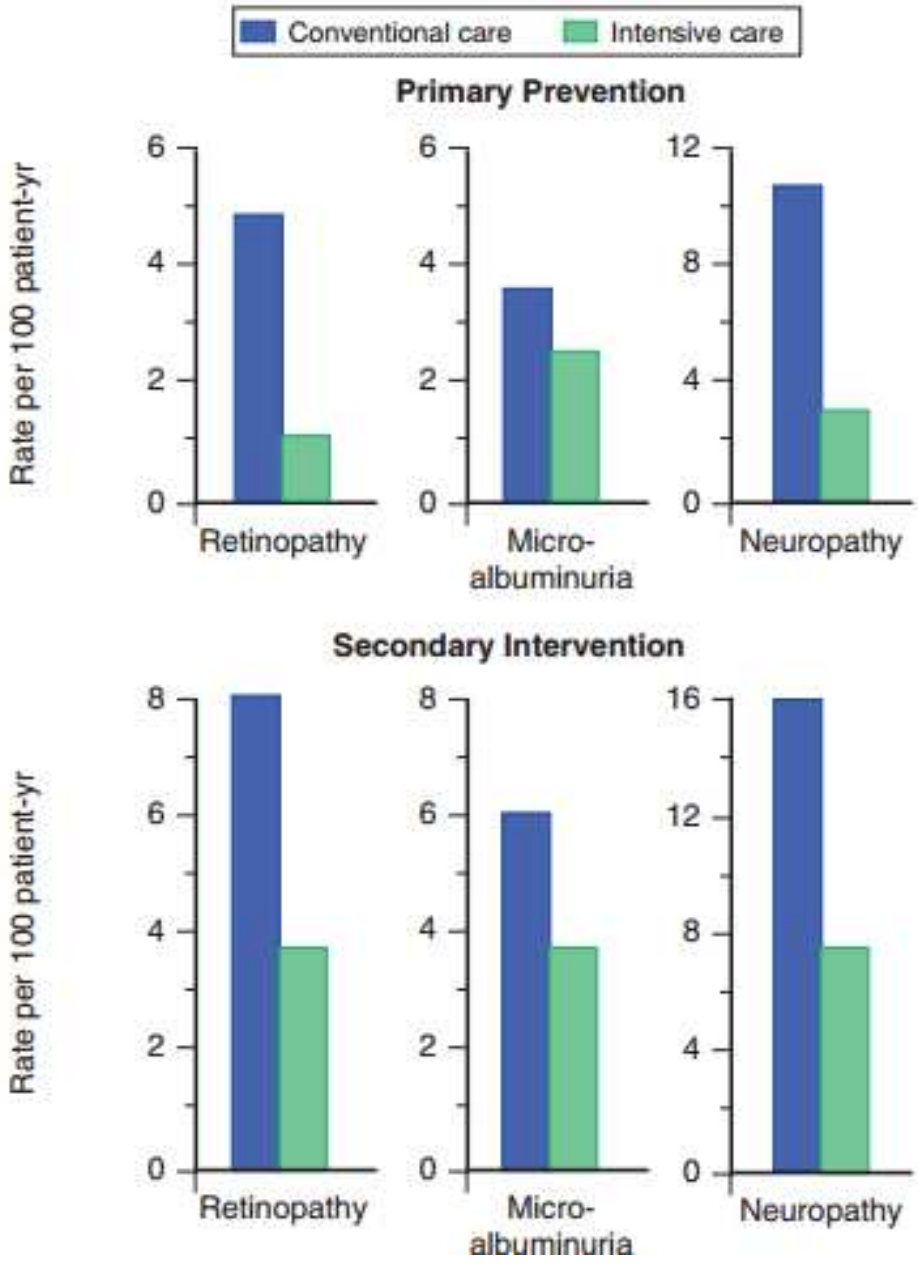


FIGURE 12: SUMMARY OF RESULT OF DCCT[63]

TREATMENT:

Replacement therapy with exogenous insulin. Is required for all patients with type 1 diabetes mellitus. At the onset of type 1 diabetes, many patients recover some pancreatic cell function and may temporarily need only low doses of exogenousinsulin to supplement their own endogenous insulin secretion. This is known as the honeymoon period. Within 8 weeks to 2 years, however,most of these patients show either absent or negligible pancreatic cell function[62].

At this point, these patients should be switched to a moreflexible insulin regimen with a combination of rapid-acting insulin analogs or regular insulin together with intermediate-acting or long-actinginsulin. At a minimum, the patient should be on a three-injection regimen and frequently may need four or more injections. Twice-daily split dose insulin mixtures cannot maintain near normalization of blood glucose without hypoglycemia (particularly at night) and are not recommended. Self-monitoring of blood glucose levels is required for determining the optimal adjustment of insulin dosage and the modulationof food intake and exercise in type 1 diabetes.

TABLE 6: DIFFERENT TYPES OF INSULIN

CLASS	PREPARATION	ONSET OF EFFECT	PEAK EFFECT (HR)	DURATION OF ACTION (HR)
Rapid acting	Lispro, aspart, or glulisine	10-15 min	1-2	3-4
Short acting	Regular (R)	30 min	2-4	5-8
Intermediate acting	NPH (N)	2-4 hr	6-12	16-24
Long acting	Glargine Detemir	2-4 hr 1 hr	No peak No peak	>24 Up to 24

Certain caveats should be kept in mind regarding insulin treatment. Considerable variations in absorption and bioavailability exist, even when the same dose is injected in the same region on different days in the same individual. Such variation often can be minimized by injecting smaller quantities of insulin at each injection and consequently using multiple injections. Furthermore, a given insulin dosage may demonstrate considerable variability in pharmacokinetics in different individuals, either because of insulin antibodies that bind insulin with different avidity or for other as yet unknown reasons. A properly educated patient should be taught to adjust insulin dosage by observing the pattern of recorded self-monitored blood glucose levels and correlating it with the approximate duration of action and the time to peak effect after injection of the various insulin preparations.

A combination of rapid-acting insulin analogs and long-acting insulin's (insulin glaring or insulin detemir) allows for more physiologic insulin replacement. In clinical studies, combinations of rapid-acting insulin analogs (insulin lispro or insulin aspart) with meals together with intermediate-acting (NPH) or longer acting insulin (insulin glargine) for basal coverage have now been shown to have improved HbA 1c values[62.]

VITAMINS:

Vitamin is derived from the Latin word *vita* =life, *Amin*=amine. It was named by casimir funk who thought that these substances were amines .The term vitamin is defined as "a group of substances that are essential, in small quantities, for the normal functioning of metabolism in the body". Vitamins are mainly divided into two groups: water soluble (vitamins B-complex and C) and fat-soluble (A, D, E and K).Unlike water-soluble vitamins that need regular replacement in the body, fat-soluble vitamins can be stored for long periods in the human body in liver and fatty tissues and are eliminated much more slowly than water-soluble vitamins.

VITAMIN B 12:

The discovery of vitamin B12 dates back to 1926, when, Murphy and Minot, based on Whipple's observation that anemic dogs could be cured by feeding them raw liver, discovered that pernicious anemia could be treated by supplementing the human diet with liver. After this observation, an intensive search for the "liver factor" was started. It was only, in 1948, when the "anti-pernicious anemia factor", which became called Vitamin B_{12}, was finally purified and isolated by Folkers and his co-workers, and by Smith and Parker. Its importance is stressed by the fact that Whipple, murphy and Minot were awarded Nobel Prize in 1934 for their discovery[64].

Vitamin B 12 is an essential co factor that plays an important role in methylation of DNA that is integral for DNA synthesis and cellular metabolism. Its deficiency affects cellular metabolism and can lead to serious clinical consequences.[1]

BIOCHEMISTRY OF VITAMIN B 12;

Vitamin B12 is water soluble, heat stable and red in color. It contains 4.35% cobalt by weight. It contains one cobalt atom. Four pyrrole rings coordinated with a cobalt atom is called a Corrin ring. . The 5th valencyof the cobalt is covalently linked to a substituted benzimidazole ring. This is then

called cobalamin. The 6thvalency of the cobalt is satisfied by any of the following groups: cyanide, hydroxyl, adenosyl or methyl.

a. Cyanocobalamin

When cyanide is added at the R position, the molecule is called cyanocobalamin. During the isolation procedure, cyanide is added to get stablecrystals. The CN group has no physiological function, it is only a laboratory artefact. Oral preparations are in this form.

b. Hydroxycobalamin

When hydroxyl group is attached at the R position, it is called hydroxycobalamin or vitamin B12a.Injectable preparations are in this form.

c. Adenosylcobalamin (Ado-B12)

When taken up by the cells, these groups are removed and deoxyadenosylcobalamin or AdoB12 is formed. This is the major storage form, seen in liver.

d. Methyl cobalamin

When the methyl group replaces adenosyl group, it is known as methyl cobalamin. This is the major form seen in blood circulation as well as in cytoplasm of cells. The Ado-B12and methyl B12are the functional co-enzymes in the body.

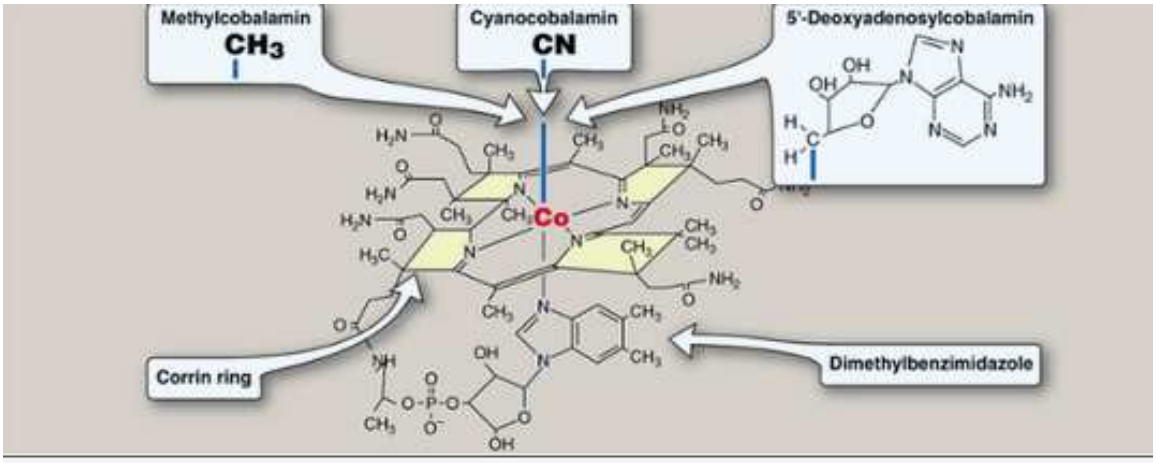


FIGURE 13: CHEMICAL STRUTURE OF VITAMIN B 12.

Biochemical functions of vitamin B12

Vitamin B 12 serves as a co factor in two important bio chemical reaction[76,79].

A) remethylation of homocysteine to form methionine.

B) Isomerization of methylmalonyl CoA.

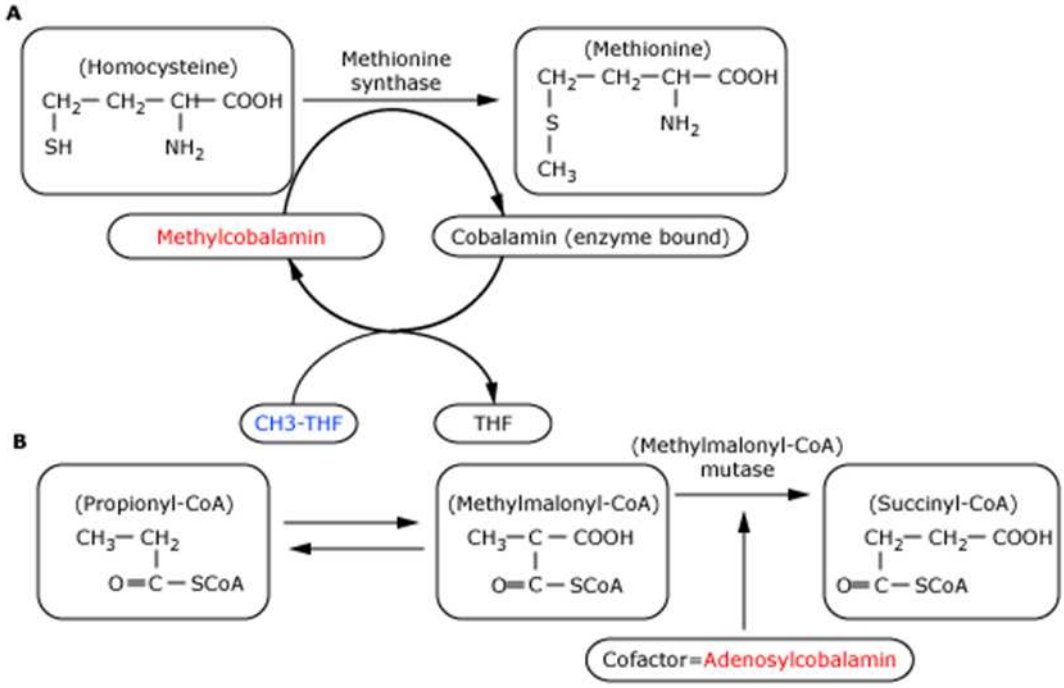


FIGURE 14:

ROLE OF VITAMIN B12 IN HOMOCYSTEINE AND METHYLMALONIC ACID METABOLISM

REMETHYLATION OF HOMOCYSTEINE TO FORM METHIONINE:

Vitamin B12 is the cofactor through which the methyl group of N5-methyl-TH4 is transferred to homocysteine to regenerate methionine. Vitamin B12 is active in its reduced form, Cobalamin. The catalytic activity of methionine synthase is regenerated by methionine synthase reductase, which uses S- adenosylmethionine to catalyze the reductive methylation of

vitamin BI2.In most cells, N5-methyl-TH4 is the only methyl donor for the synthesis of methyl- B 12.

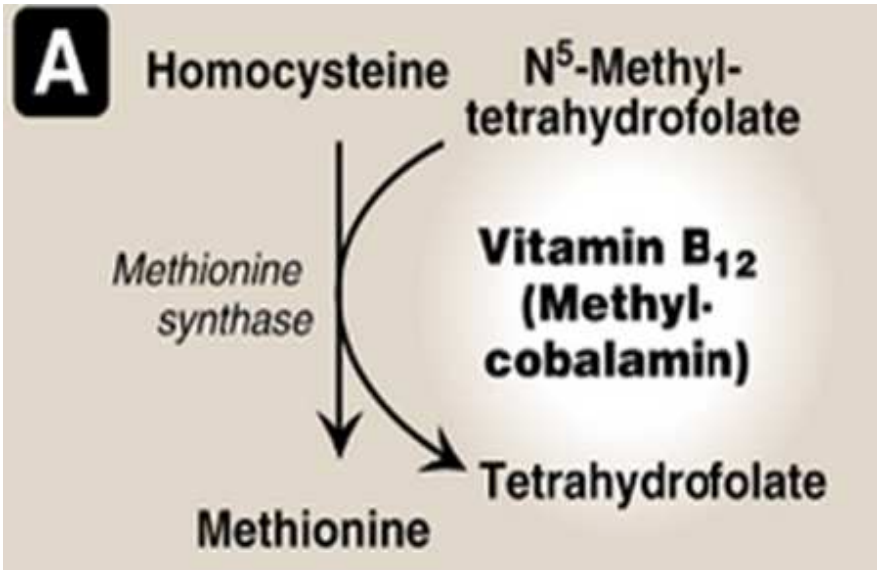


FIGURE 15:

CONVERSION OF HOMOCYSTEINE TO METHIONINE

ISOMERIZATION OF METHYLMALONYL COA:

Deoxyadenosyl-B 12 is a cofactor for methylmalonyl-CoA mutase, which catalyzes the conversion of methylmalonylcoA to succinylcoA. This reaction is a key component of the pathway by which the carbon skeleton of

Propionyl-CoA is metabolized. Propionyl-CoA is generated when odd-chain fatty

Acids are oxidized and during the catabolism of the carbon skeletons of valine,

Isoleucine, and cysteine. Subsequent metabolism of succinyl-CoA by means of the TCA cycle generates oxaloacetate, which can be used to synthesize glucose.

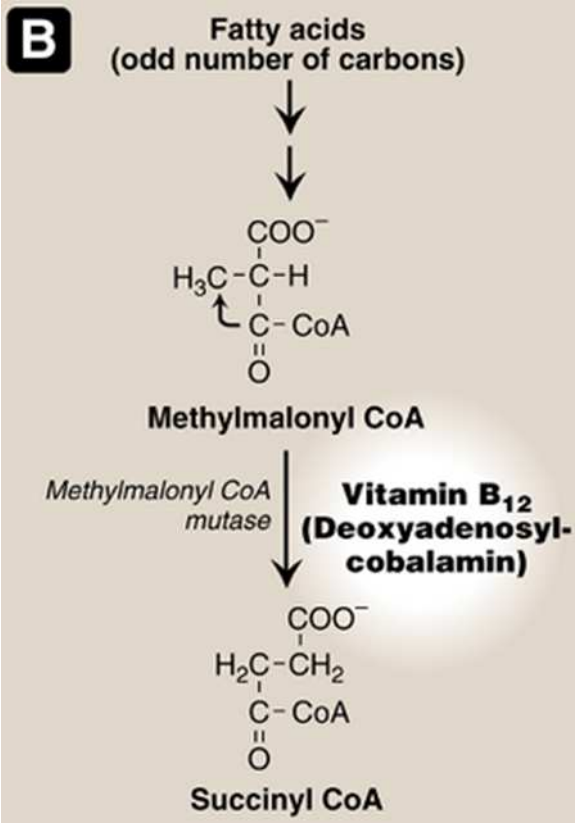


FIGURE 16: ISOMERIZATION OF METHYL MALONYL COA

PHYSIOLOGY OF VITAMIN B12 [66];

For adults, the Recommended Dietary Allowance for Cobalamin is 2 mcg/day, for pregnant and lactating women, the RDA is 2.6 mcg/day. For children, the RDA for Cbl ranges from 0.7 mcg/day during the toddler stage to 2 mcg/day during adolescence. Similarly, if a patient with successfully treated pernicious anemia stops taking B12, it will take the same time (approximately three years) for the deficiency to recur.

Utilization of dietary vitamin B12 is dependent on both gastric HC1 and two specialized proteins, R proteins and intrinsic factor. Dietary vitamin B 12 is covalently bound to polypeptides; release of vitamin B 12 normally occurs in the stomach through the combined hydrolytic actions of HC1 and pepsin. R proteins (also designated huptocorrins or cobalophilins) are present in both saliva and gastric juice. They bind the vitamin B12 prior to its release from the polypeptides, and remain associated with vitamin B12 until the R proteins are hydrolyzed in the small intestine.

Intrinsic factor (IF), a glycoprotein produced by the parietal cells of the stomach, is essential for the absorption of vitamin B12. Intrinsic factor is so named because early studies demonstrated that both a dietary (extrinsic) factor and a protein produced by the normal stomach (intrinsic) were necessary for the prevention of pernicious anemia. As soon as vitamin Bl2 is released from the R proteins, it binds to intrinsic factor. The IF-B 12 complex is then recognized by specific receptors, called cubulins, located primarily in the distal ileum. The major protein that transports vitamin B12 from the intestine to other tissues is transcobalamin 11, which is a member of the R-protein family [66, 67,73].

Electron microscopic studies have shown colocalization of cubilin with the endocytic proteins megalin and "amnionless" (AMN), which may mediate vesicular trafficking of the complex via a calcium-dependent

mechanism. It is likely that the functional ileal receptor for the cobalamin-intrinsic factor complex is a complex of AMN and cubilin.

Mutations in either the cubilin or the AMN gene cause hereditary megaloblastic anemia, while absence of megalin has been associated with failure of normal renal tubular reabsorption of the Cbl-transcobalamin complex in mice.

Thus, absorption of cobalamin depends upon five factors,

- Adequate dietary intake
- Acid-pepsin
- Pancreatic proteases
- functional intrinsic factor(in stomach)
- functioning Cbl-IF receptors (in ileum)

The need for an intact upper GI tract for effective absorption of cobalamin was shown in a report of patients receiving a Roux-en-Y gastric bypass for morbid obesity. Standard multivitamin preparations were inadequate to maintain vitamin B12, folic acid, iron, calcium, and vitamin D levels[80-84.]

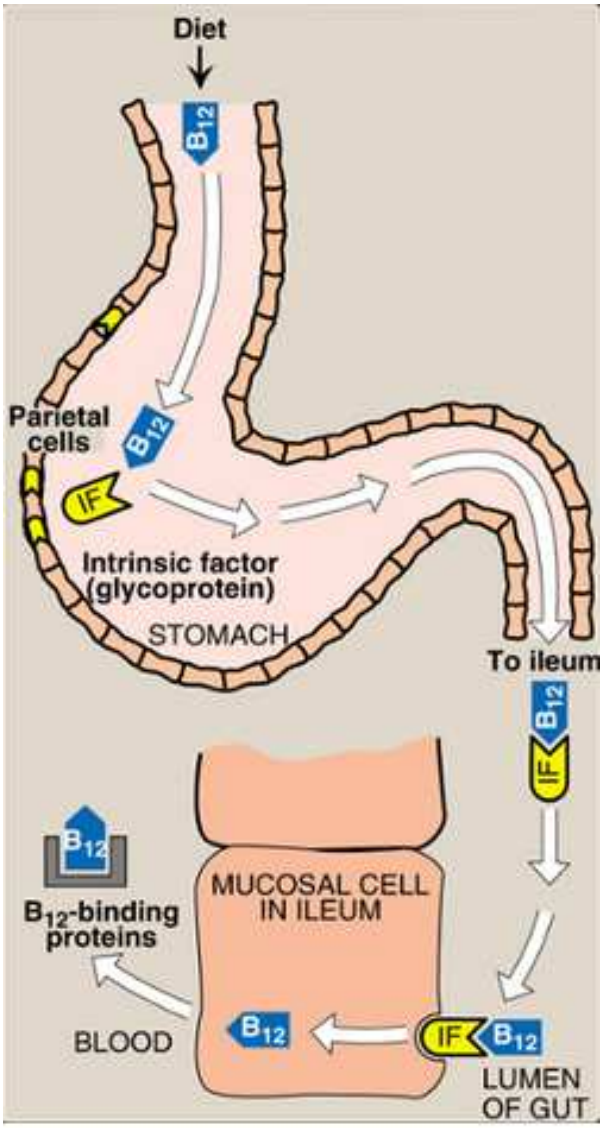


FIGURE 17: ABSORPTION OF VITAMIN B 12

After being taken up by ileal enterocytes, Cobalamin is exported via the ATP-binding cassette (ABC)-drug transporter ABCC1 (also called multidrug resistance protein, MRP1), present in the basolateral membrane of intestinal epithelium and other cells. Cobalamin enters plasma bound to three transcobalamines: TC I, II, and III. Up to 80 percent of Cobalamin is bound to TC I and III, which have no identified role in Cobalamin metabolism. It is

the TC II-Cbl complex that is physiologically important. The three-dimensional structure of transcobalamin shows that there are two domains for binding cobalamin. This complex has a half-life of six to nine minutes and binds to specific cell surface receptors from which it enters cells by receptor mediated endocytosis. cobalamin in the cells is metabolized into two coenzymes: adenosyl-Cbl; and methyl-Cbl, the functions of which are described above.

Transcobalamin II has a common polymorphism, in which proline replaces arginine at codon 259. In one study, normal older adults homozygous or heterozygous for the proline polymorphism had higher levels of HoloTCII and lower levels of methylmalonic acid than those homozygous for the arginine form, suggesting that the former may be more efficient in delivering cobalamin to tissues . It is not known whether the TCII genotype influences susceptibility for development of clinical manifestations of cobalamin deficiency.

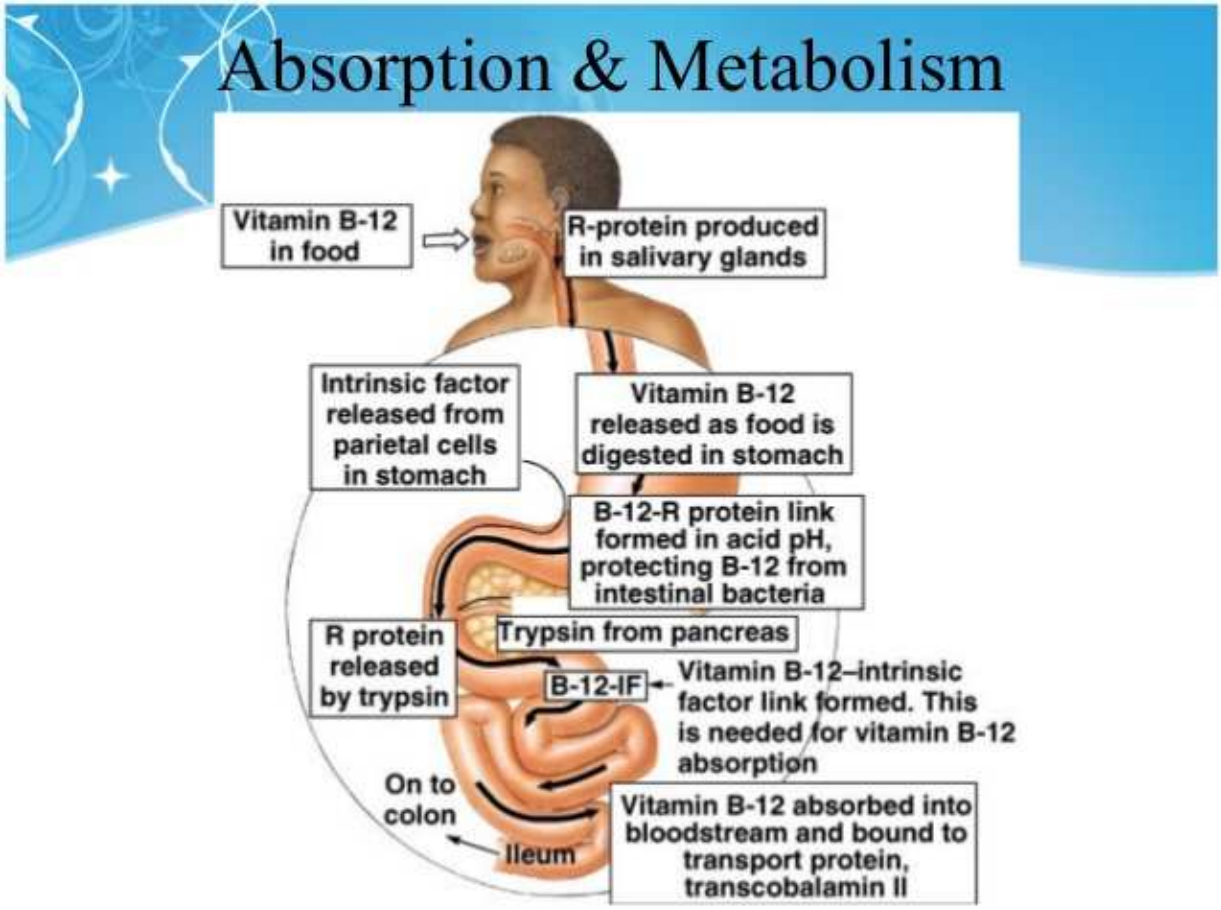


FIGURE 18: ABSORPTION AND METABOLISM OF VITAMIN B 12

CAUSES OF VITAMIN B 12 DEFICIENCY[73-77];

Impaired gastric absorption

•Pernicious anemia

• Gastrectomy—partial or total

• Zollinger-Ellison syndrome

Impaired intestinal absorption;

• Ileal resection or disease—for example, Crohn's inflammatory bowel disease and tuberculous ileitis

• Blind loop syndrome

• Luminal disturbances: chronic pancreatic disease and gastrinoma

• Parasites: giardiasis, bacterial overgrowth, and fish tapeworm

Pancreatic insufficiency

Decreased intake

• Malnutrition

• Reduced intake of animal products

• Strict vegan diet

Congenital/inherited

• Intrinsic factor receptor deficiency/defect—Imerslund-Gräsback syndrome

• Congenital deficiency of intrinsic factor—“juvenile” pernicious anemia

• Cobalamin mutation (C-G-1 gene)

• Transcobalamin deficiency

Increased requirements

• Hemolysis

• HIV

Drugs

• Alcohol

• Nitrous oxide

• Proton pump inhibitors

• H 2 receptor antagonists

• Metformin

• Colchicine

• Slow K (potassium chloride) preparations

• Cholestyramine

CLINICAL FEATURES OF VITAMIN B 12 DEFICIENCY;

The clinical manifestations of vitamin B12 deficiency represent the effects of depletion on multiple systems and vary greatly in severity. The clinical manifestations are heterogeneous but can also be different depending on the degree and duration of deficiency[89, 90,91].

Mild deficiency manifests as fatigue and anemia, with indices suggesting B 12 deficiency but an absence of neurological features. Moderate deficiency may include an obvious macrocytic anemia with, for example, glossitis and some mild or subtle neurological features, such as distal sensory impairment.

Severe deficiency shows evidence of bone marrow suppression, clear evidence of neurological features, and risk of cardiomyopathy. However, it is important to recognize that clinical features of deficiency can manifest without anemia and also without low serum vitamin B12 levels. In these cases treatment should still be given without delay.

Bone marrow

The bone marrow is most commonly affected. Anemia may range from mild to severe, with symptoms of fatigue on exertion, dyspnea, palpitations, and pallor. All cell lines can be affected, with macrocytic anemia, low white cell count or neutropenia, and thrombocytopenia.

Tissues and organ dysfunction

Epithelial changes with vitamin B12 deficiency include skin hyperpigmentation and glossitis. Reproductive tissue can be affected, manifesting as infertility. Deficiency can also result in osteoporosis, with reduced bone derived alkaline phosphatase and plasma osteocalcin. Rarely, cardiomyopathy can occur.

Neurological features

Neurological impairment includes motor disturbances, sensory loss, abnormal balance and reflexes, cognitive impairment, and memory loss. Extreme cases may present with stupor or psychosis. An estimated 20% of patients with neurological signs do not manifest anemia. Clinical features of anemia may be minimal and the blood indices may not reflect important anemia. Neurological symptoms can occur in isolation so it is important to consider a diagnosis of vitamin B 12 deficiency in the presence of

neurological symptoms of unknown cause, as neurological features may progress and become irreversible.

Sub-acute combined degeneration of the spinal cord involves demyelination of the posterior and lateral tracts. Initial bilateral peripheral neuropathy can progress to axonal degeneration and neuronal death if left untreated. This is followed by disturbances of proprioception, vibratory sense, and areflexia. Patients may mention clumsiness, poor coordination, and difficulty walking. Without treatment, weakness and stiffness may develop, manifesting as spastic ataxia. Damage to peripheral nerves results in sleepiness, altered taste and smell, and optic atrophy. In severe deficiency or advanced stages, a dementia-like illness may be seen, and frank psychosis with hallucinations, paranoia, and severe depression.

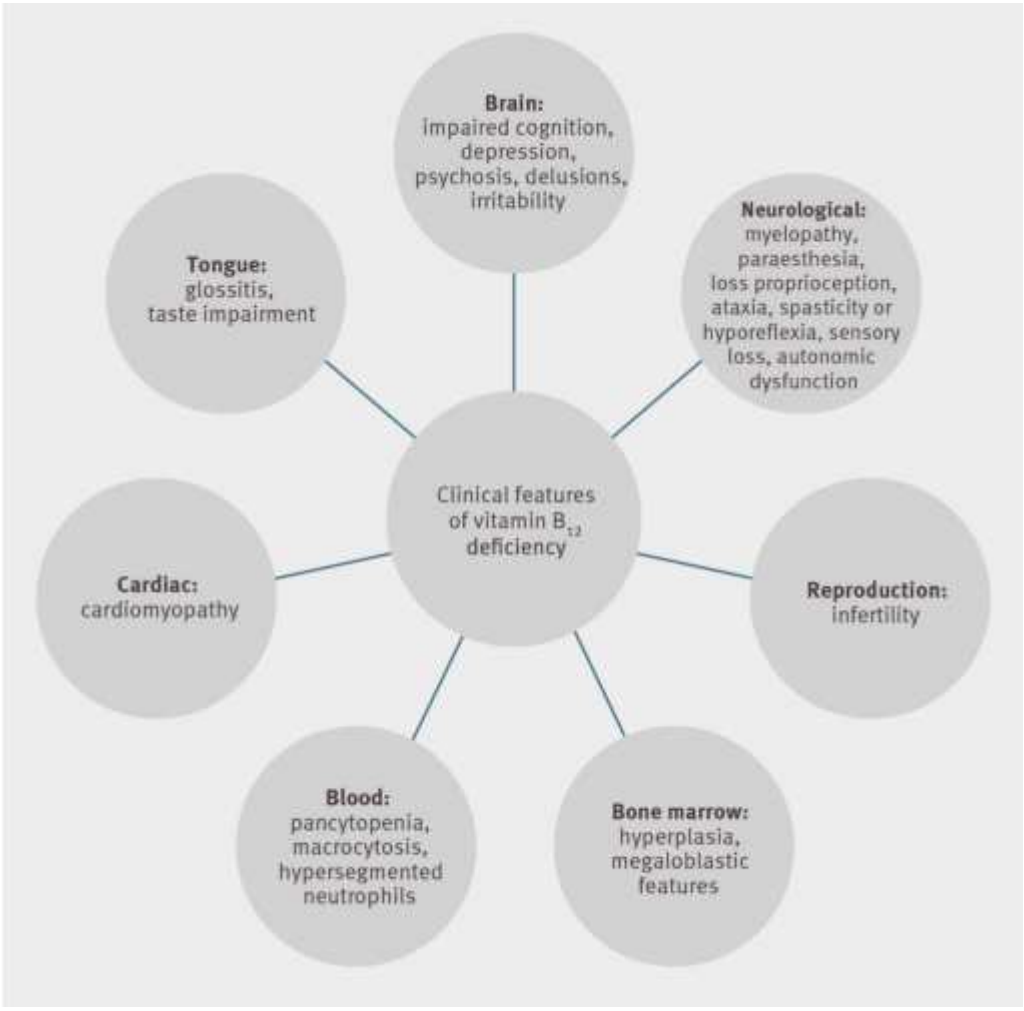


FIGURE 19: CLINICAL FEATURE OF VITAMIN B12 DEFICIENCY

TABLE 7: LABORATORY ASSESSMENT OF VITAMIN B12[94,95]:

Assessment and investigation	**FINDING**
Mean cell volume	Normal or increased
Haemoglobin	Normal or low
Reticulocyte count	Low
Lactate dehydrogenase	Increased
Peripheral smear	Anaemia, macrocytosis hyper segmented neutrophils (>5% of neutrophils with=5 lobes).Neutropenia and thrombocytopenia
Bone marrow aspirate and trephine	Hyper cellular marrow, decreased myeloid: erythroidratio.Nuclear chromatin is diffuse and immature. White cells show megaloblastic features with large band forms and giant metamyelocytes. Nuclei may have abnormal stain appearance. Megakaryocytes may have increased nuclear lobulation or hypogranulation
Serum/plasma cobalamin concentration	Low (<150 ng/l)
Serum/plasma holotranscobalamin concentration	Low (<5 pmol/l)
Serum/plasma or urine methyl malonic acid	Increased (>350 nmol/l)
Plasma homocysteine	Raised (>15 micromol/l)

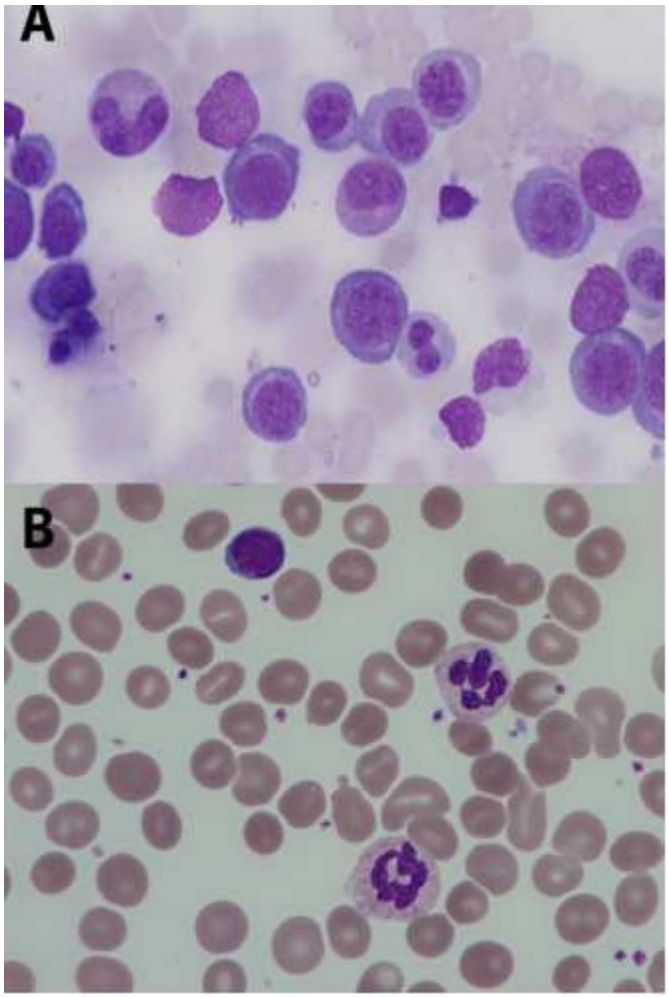


FIGURE 20:

BLOOD AND BONE MARROW ASPIRATE INDICATING

MEGALOBLASTIC ANEMIA

VITAMIN B 12 LEVEL;

Vitamin B 12 level is actually a measurement of serum cobalamin. Measurement of vitamin B 12 in serum is the most common assay used to evaluate vitamin B 12 levels. The test, however, also measures both serum holohaptocorrin and serum holotranscobalamin, and as such may mask true deficiency or falsely imply a deficient state. The test is widely available at low cost and uses an automated method and competitive-binding immune chemiluminescence. The clinically normal level for serum cobalamin is not completely clear. It has been suggested that serum cobalamin<148 pmol/L (200 ng/L) would be sensitive enough to diagnose 97% of patients with vitamin B deficiency. It is not clear what level of serum cobalamin may represent subclinical deficiency.

Identifying the cause of vitamin B 12 DEFICIENCY

Once a diagnosis of vitamin B 12 deficiency is identified, history taking and examination are important. If there is no obvious dietary lack of vitamin B or malabsorption, tests for intrinsic factor and antiparietal cell antibodies should be performed to exclude pernicious anemia.

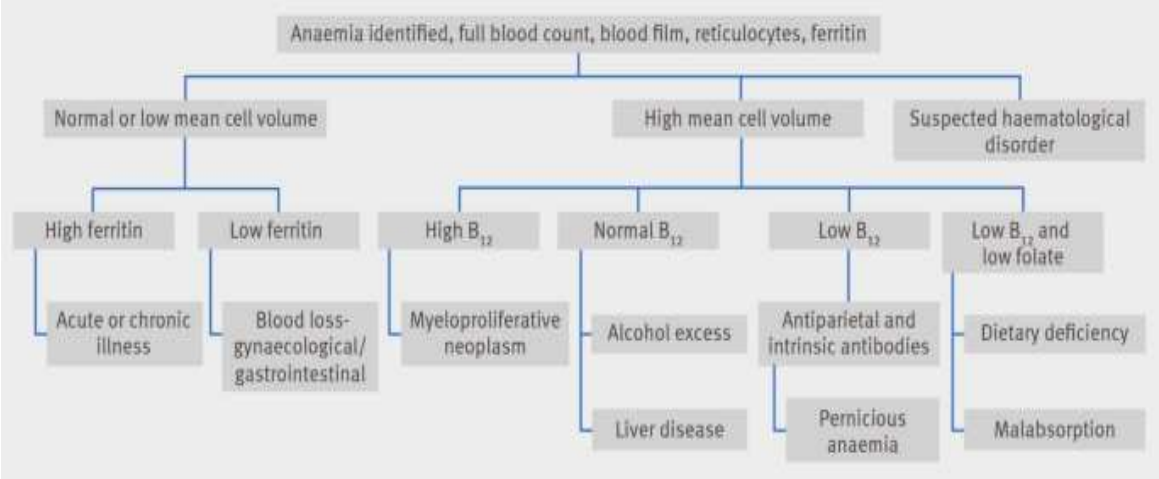


FIGURE 21: DIAGNOSTIC ALGORITHM FOR VITAMIN B12 DEFICIENCY

PERNICIOUS ANEMIA:

Pernicious anemia is a severe megaloblastic anemia that results from inadequate tissue levels of vitamin B12. The underlying problem is a lack of intrinsic factor production by the stomach. Pernicious anemia is due principally to an autoimmune gastritis in which the blood contains antibodies against intrinsic factor and other proteins of the parietal cells. These antibodies damage the patient’s mucosa and abolish the secretion of both intrinsic factor and HC1.

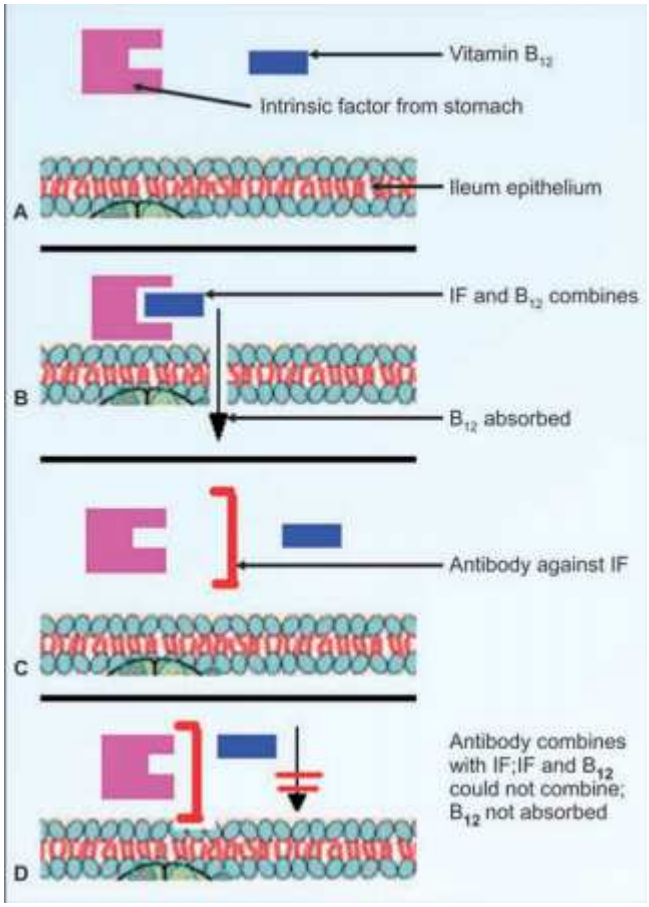


FIGURE 22: PERNICIOUS ANEMIA

(A) Intrinsic factor secreted from stomach reaches intestine.

(B) = Vitamin B12 absorbed with the help of intrinsic factor.

(C) = In pernicious anemia, antibody against IF is produced.

(D) = In presence of antibody, absorption is not taking place

The hematological presentation of pernicious anemia is indistinguishable from that of folic acid deficiency. Vitamin B12 deficiency results in an inability to transfer the methyl group from 5-methyl-TH4 to homocysteine to form methionine. Since the methyl group of 5-methyl-TH4 cannot be oxidized to 5', 10'-methylene-TH4 or other one-carbon folate derivatives, the TH4 pool becomes "trapped" as 5-methyl-TH4 in vitamin B 12-deficient persons. This, in turn, diminishes the availability of TH4 for nucleotide synthesis, resulting in megaloblastic anemia. With time, pernicious anemia can result in progressive neurological degeneration, which in its later stages may present with tingling or numbness of the extremities and diminished reflexes. Neurological damage may be due to insufficient 5'-deoxyadenosyl-B 12 to support the methylmalonyl-CoA mutase reaction, resulting in a buildup of methylmalonic acid. When present at elevated concentrations, methylmalonyl-CoA can substitute for malonyl-CoA in fatty acid synthesis, leading to the synthesis of branched-chain fatty acids, which are incorporated into phospholipids of the myelin sheath. Alternatively, the neurological symptoms may be due to inadequate amounts of S-adenosylmethionine in neural tissues.

TREATMENT:

TIMING OF TREATMENT;

It is usually acceptable to start treatment within a few days of a confirmed diagnosis. If there are neurological disturbances then treatment should be expedited and started without delay. Specialist input should be sought in the event of neurological features, including impaired cognitive state. Neurological presentation may occur in the absence of hematological changes, with early treatment essential to avoid permanent neurological disability. Emergency treatment with packed red cell transfusion may be required for major anemia in the presence of cardiovascular compromise[82,86].

Parenteral treatment;

Data from randomized controlled trials and observational studies for parenteral treatment are lacking; however, the expert For standard treatment is to begin parenteral treatment with intramuscular hydroxocobalamin. This bypasses the possibility of the debate about whether the treatment will be adequately taken, absorbed, and metabolized. Standard initial treatment for patients without neurological involvement is 1000 µg intramuscularly three times a week for two weeks. If there are neurological symptoms then 1000 µg intramuscularly on alternate days should be continued for up to three weeks or until there is no further improvement. In irreversible cases, for example,

pernicious anemia, the treatment should be continued for life. For temporary causes, such as pregnancy, the treatment can be reviewed when the patient is fully replete and the causative agent removed. Hydroxocobalamin is generally well tolerated. Rarely, side effects include itching, exanthema, chills, fever, hot flushes, nausea, dizziness, and very rarely anaphylaxis. There can be a Cross over reaction to cobalamin; if there is concern about this then the drug should be administered in a place where hypersensitivity can be managed, with hydrocortisone and chlorphenramine cover available.

Oral treatment;

Cyanocobalamin is an oral preparation that can be given at a Dose of 50-150 µg daily. The duration is determined by the causeof the deficiency. If the cause is irreversible then parenteral therapy should be continued for life. This is a drug preparation requiring conversion to metabolically active cobalamins. A

Cochrane review of two randomized controlled trials in 108 people with vitamin B deficiency found that high oral doses of B 12 (1000 µg and 2000 µg daily) were as effective as intramuscular treatment in achieving hematological and neurological responses. However, UK national consensus is that there are arguments against the use of oral cobalamin in severely deficient patients and those with malabsorption. High dose oral cobalamin

may be a suitable alternative in selective cases, where intramuscular injections are not tolerated and compliance is not a problem, as previously described.

Oral treatment may be considered in certain situations—for example, in mild or subclinical deficiency with no clinical features and when absorption and compliance are definitely not a problem.

Treatment with vitamin B leads to the production of new Erythrocytes, which results in an intracellular influx of potassium. This may produce severe hypokalemia, which requires monitoring and appropriate treatment.

PREVENTION OF VITAMIN B 12 DEFICIENCY;

It is not currently possible to prevent vitamin B 12 deficiency from pernicious anemia. Deficiency due to gastric and terminal ileum disease should be anticipated and supplemented before clinical presentation of deficiency. Breakfast cereals are fortified with vitamin B 12, as a non-animal based dietary source. This may be useful for older people (>65 years) and those with a restricted diet. Each portion contains approximately 25% of the recommended daily intake of vitamin B 12.For patients taking long term metformin and proton pump inhibitors the use of oral cyanocobalamin could be considered, or increased screening and vigilance of vitamin B 12 deficiency.

MATERIALS AND METHODS

MATERIALS AND METHODS

STUDY DURATION:

This study was conducted over a period of six months.

STUDY POPULATION:

The study comprised of type 1 Diabetes Mellitus patients visiting the Outpatient department of institute of diabetology, Rajiv Gandhi government general hospital, Chennai. Age and sex matched healthy volunteers served as controls.

Sample Size:

Total Number of Subjects	:	100
Number of controls	:	50
Number of Type 1 Diabetes Mellitus cases	:	50

TYPE OF STUDY:

Observational study

ETHICS COMMITTEE APPROVAL:

Obtained.

INCLUSION CRITERIA:

- Patients diagnosed as Type 1 Diabetes Mellitus

EXCLUSION CRITERIA:

- Type 2 diabetes mellitus
- pregnancy
- liver disorders
- renal failure
- hypothyroidism
- Patients on drugs which alters serum vitamin b 12 levels
- Previous gastro intestinal surgery.
- Patients with established vitamin b 12 deficiency.

DATA COLLECTION AND METHODS

METHODOLOGY

Data for the study was collected from all those who fulfilled the inclusion and exclusion criteria after taking a detailed case history and obtaining a written informed consent.

METHOD OF COLLECTION OF DATA:

Baseline data including age and sex, detailed medical history including conventional risks factors, clinical examinations and relevant investigations were included as part of the methodology. For all the subjects standing height and weight were measured.

SPECIMEN COLLECTION:

Blood:

5 ml plain venous blood sample after overnight fasting was obtained by venipuncture for estimation of vitamin b 12 and anti-intrinsic factor anti-bodies. For PPBS, blood sample was collected 2 hours after the breakfast. For HbA1C estimation 2 ml of EDTA blood sample was collected. This was followed by centrifugation and then sample was processed immediately after collection.

DETERMINATIONS:

1. Serum vitamin B 12 level by chemiluminiscence method using the lab cut off value of 180 pmol/l .

2. Glycosylated haemoglobin by immunoturbidimetric method.

3. Fasting and post prandial blood sugars by oxidase / peroxidase method.

4. Anti-intrinsic factor antibodies by chemiluminescence method.

All the data will be entered in proforma(enclosed).

The statistical software using SPSS was used for the analysis of data and MS word and Excel have been used to generate graphs, tables etc.

OBSERVATION AND RESULTS

OBSERVATION AND RESULTS

The present study was done to evaluate the prevalence of vitamin B 12 deficiency in type 1 diabetes mellitus and try to find out cause of vitamin B12 deficiency by determining anti-intrinsic factor antibodies. 50 cases of type 1 diabetic cases were considered for this study and 50 age and sex matched healthy individuals were chosen as controls. The mean age of control and cases are presented in table 1A. A sex wise distribution of control and study groups are shown in table 1B.

AGE DISTRIBUTION:

In our study among both the cases and control group, 22 % were in the age group of 10-19 years,42% were in the age group of 20 -29 years ,28 % were in the age group of 30 -39 years and 8 % were in the age group of 40 – 49 years. The mean age is 27.5 in both the groups.

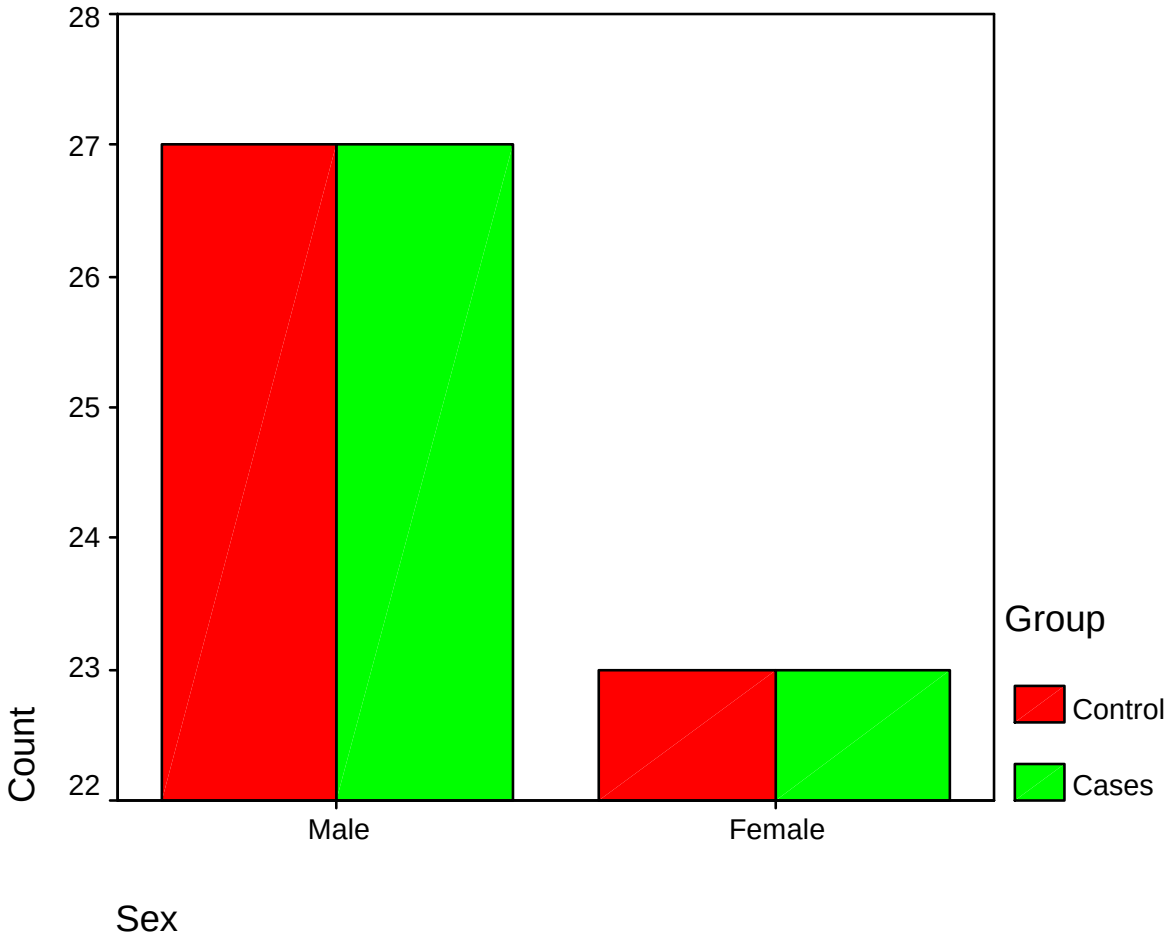
	Group	**numbers**	**Mean**	**Std. Deviation**	**P value**
AGE (years)	Control	50	27.50	8.021	1.000 **Not significant**.
	Cases	50	27.50	8.021	

SEX DISTRIBUTION:

In our study, among both cases and controls, 54 % were males and 46 % were female.

			Group		Total	P value
			Control	Cases		
Sex	Male	Count	27	27	54	1.000 Not significant
		% within Sex	50.0%	50.0%	100.0%	
		% within Group	54.0%	54.0%	54.0%	
	Female	Count	23	23	46	
		% within Sex	50.0%	50.0%	100.0%	

SEX DISTRIBUTION IN CASES AND CONTROLS



VITAMIN B 12 DEFICIENCY IN CASES AND CONTROLS:

Among the type 1 diabetes group, 24 patients had vitamin B 12 deficiency (< 180 pmol/l), and 26 patients had normal vitamin B 12 levels (>180 pmol/l).

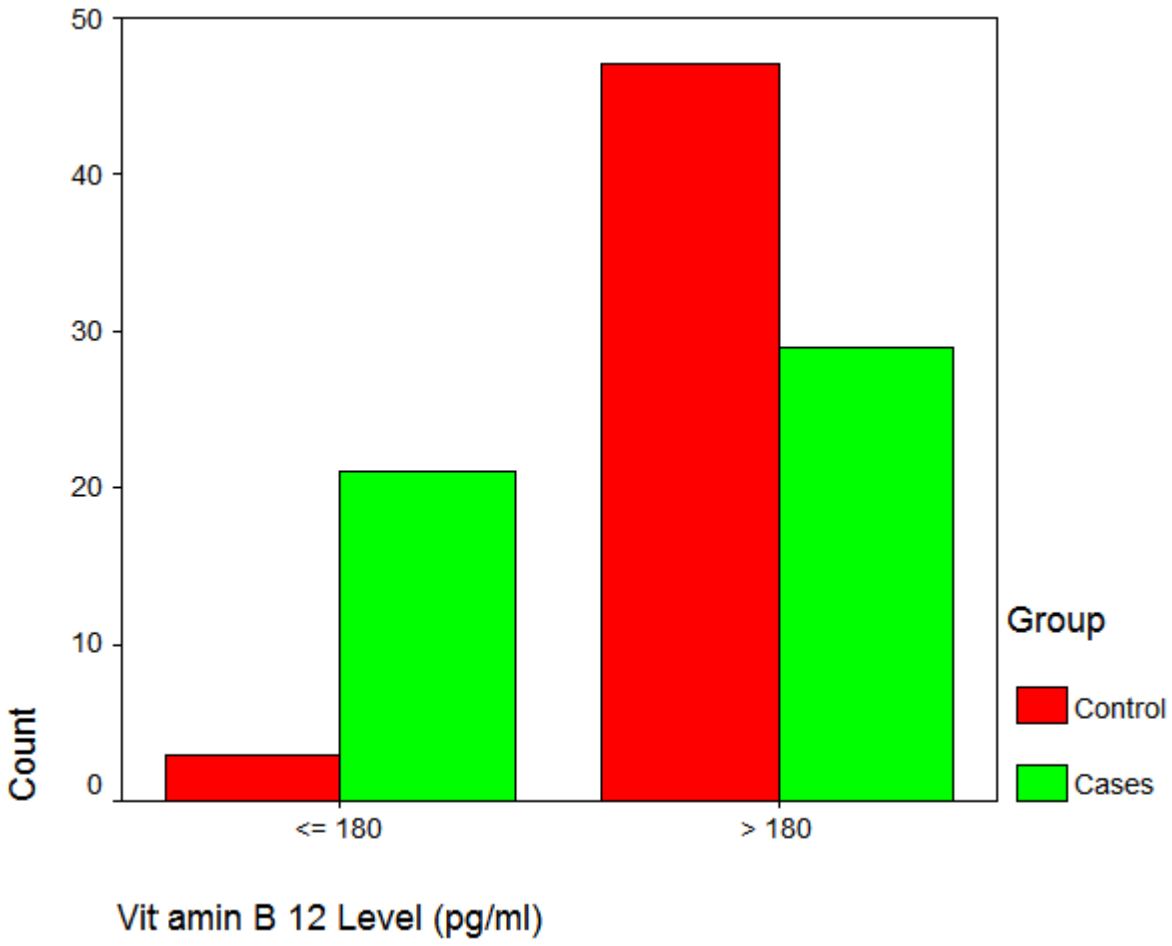
Among the controls, 3 patients had vitamin B 12 deficiency (< 180 pmol/l), and 47 patients had normal vitamin B 12 levels (>180 pmol/l).

The correlation of vitamin B 12 levels between these groups is highly significant (p<0.001).

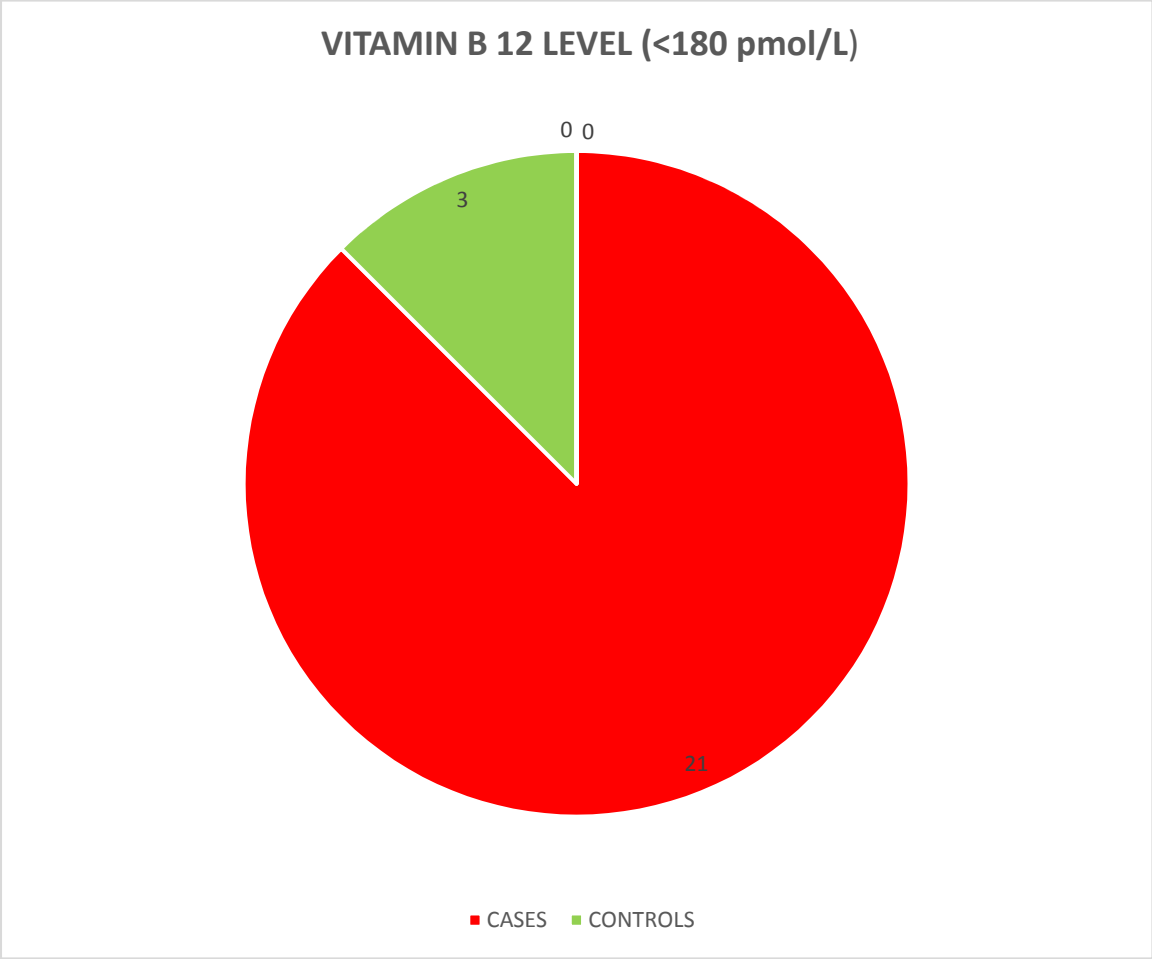
Group		**N**	**Mean**	**Std. Deviation**	**P value**
Vitamin B 12 Level (pmol/l)	Control	50	308.00	71.394	<0.001 **HIGHLY SIGNIFICANT**
	Cases	50	227.70	75.697	

			Group		Total	
			Control	**Cases**		**P VALUE**
Vitamin B 12 Level (pg/ml)	<= 180	Count	3	21	24	<0.001 **HIGHLY SIGNIFICANT**
		% within Vitamin B 12 Level (pmol/l)	12.5%	87.5%	100.0%	
		% within Group	6.0%	42.0%	24.0%	
	> 180	Count	47	29	76	
		% within Vitamin B 12 Level (pmol/l)	61.8%	38.2%	100.0%	
		% within Group	94.0%	58.0%	76.0%	

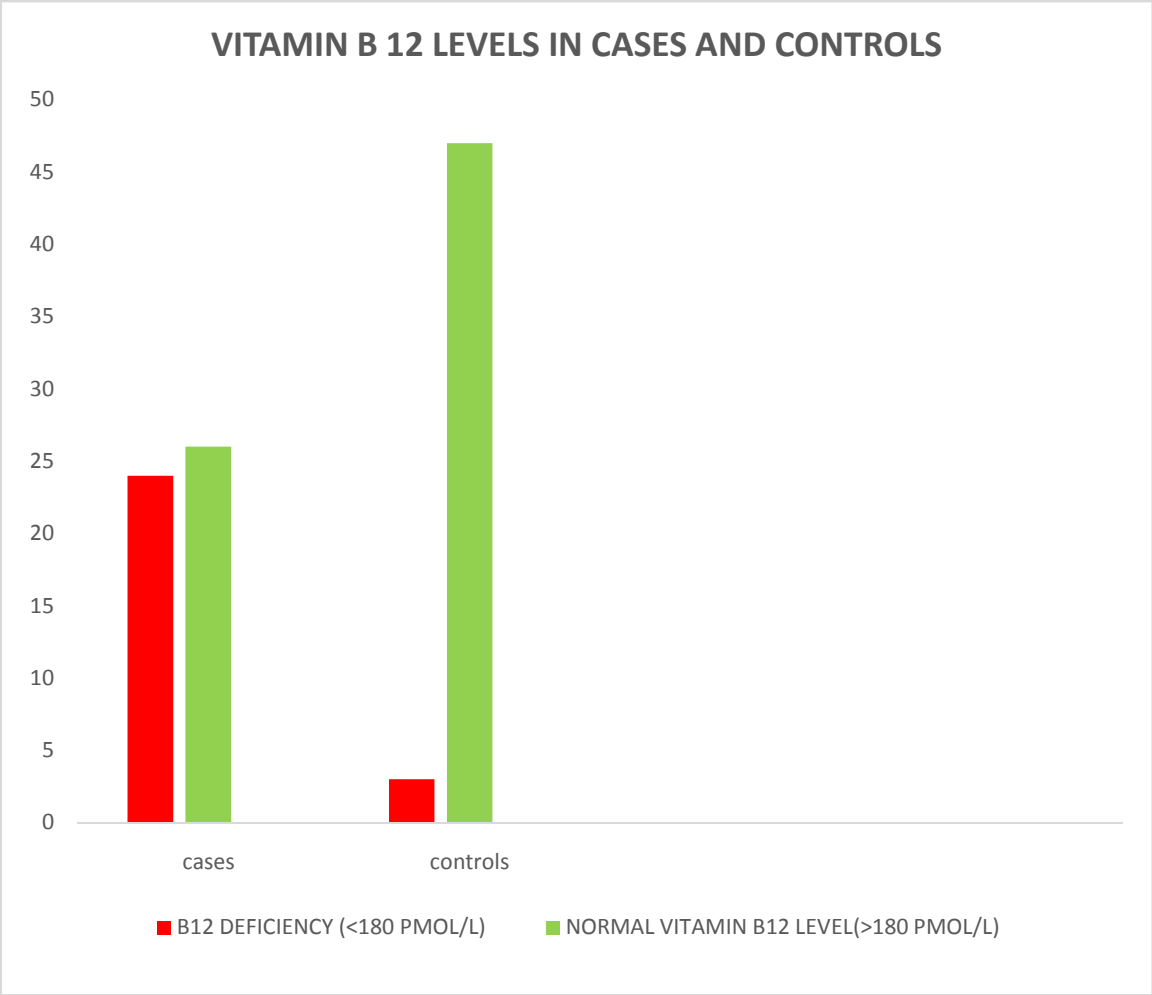
VITAMIN B 12 LEVELS CASES AND CONTROLS



VITAMIN B 12 DEFICIENCY IN CASES AND CONTROLS



VITAMIN B 12 DEFICIENCY IN CASES AND CONTROLS



ANTI INTRINSIC FACTOR ANTIBODIES IN INDIVIDUALS DEFICIENT IN VITAMIN B 12 (<180 pmol/L):

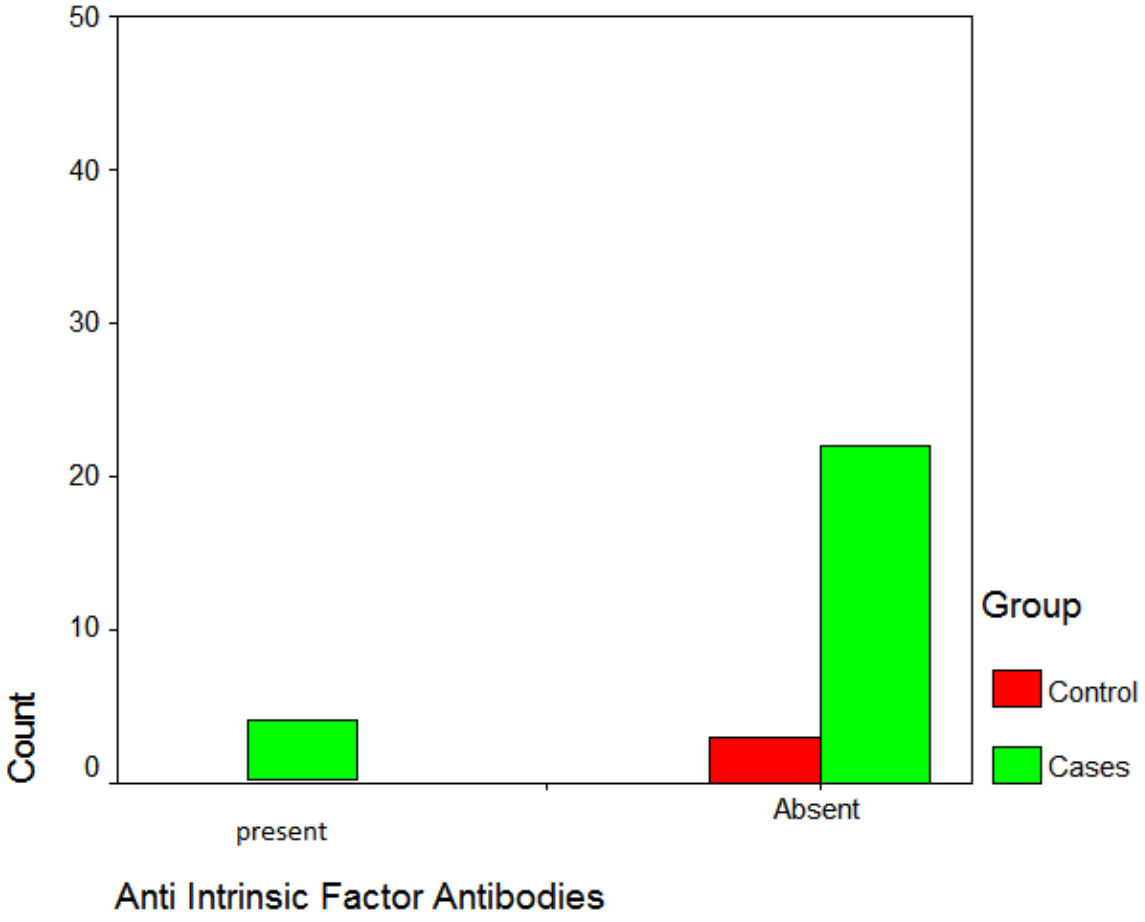
Among 24 type 1 diabetes mellitus patients who were deficient in vitamin B 12 (<180 pmol/l), 4 patients had anti intrinsic factor antibodies. Among 3 individuals in the control group, who were deficient in vitamin B 12 (<180 pmol/l), none of them had anti intrinsic factor antibodies.

The correlation between these two groups is highly significant (p<0.001).

			Group		**Total**	**P VALUE**
			Control	**Cases**		
ANTI INTRINSIC FACTOR ANTI BODIES	PRESENT	Count	0	4	4	<0.001 **HIGHLY SIGNIFICANT**
		% within Group	.0%	8.0%	4.0%	
	ABSENT	Count	3	22	25	
		% within Group	6.0%	44.0%	25.0%	

ANTI INTRINSIC FACTOR ANTIBODIES IN

CASES AND CONTROLS



DURATION OF DIABETES AND VITAMIN B 12:

Among the type 1 diabetes mellitus group, the mean duration of diabetes in those deficient in vitamin b 12 (<180 pmol/l),was 9.43 years . The mean duration of diabetes in years in those with normal vitamin b 12 (>180 pmol/l) was 8.31 years. p value (0.074 was not statistically significant).

	Vitamin B 12 Level (pmol/l)	**N**	**Mean**	**Std. Deviation**	**P VALUE**
Duration of Diabetes in years	<= 180	21	9.43	5.609	.074 Not significant
	> 180	29	8.31	6.136	

FASTING BLOOD SUGAR AND VITAMIN B12:

Among the type 1 diabetes mellitus group, the mean fasting blood sugar in those deficient in vitamin b 12 (<180 pmol/l),was 133.14 mg /dl. The mean fasting blood sugar in those with normal vitamin b 12 (>180 pmol/l) was 144.86 mg/dl. p value (0.044 was not statistically significant).

	Vitamin B 12 Level (pmol/l)	**N**	**Mean**	**Std. Deviation**	**P VALUE**
FBS (mg/dl)	<= 180	21	133.14	49.331	.044 Not significant
	> 180	29	144.86	40.639	

POST PRANDIAL BLOOD SUGAR AND VITAMIN B12:

Among the type 1 diabetes mellitus group, the mean post prandial blood sugar in those deficient in vitamin b 12 (<180 pmol/l),was 218.90 mg /dl. The mean post prandial blood sugar in those with normal vitamin b 12 (>180 pmol/l) was 239.76mg/dl. p value (0.093 was not statistically significant).

PPBS (mg/dl)	**Vitamin B 12 Level (pmol/l)**	**N**	**Mean**	**Std. Deviation**	**P VALUE**
	<= 180	21	218.90	75.845	.093 Not significant
	> 180	29	239.76	56.381	

HB A 1C AND VITAMIN B12:

Among the type 1 diabetes mellitus group, the mean HBa1c in those deficient in vitamin b 12 (<180 pmol/l), was 7.00. The mean HBa1c in those with normal vitamin b 12 (>180 pmol/l) was 6.993. P value (0.140 was not statistically significant).

HbA1C in percentage	**Vitamin B 12 Level (pmol/l)**	**N**	**Mean**	**Std. Deviation**	**P VALUE**
	<= 180	21	7.000	.3619	-.014 Not significant
	> 180	29	6.993	.2618	

DISCUSSION

DISCUSSION

Vitamin B12, an essential micronutrient which is required for optimal hemopoetic, neuro-cognitive and cardiovascular function. High prevalence of Biochemical and clinical vitamin B12 deficiency have been demonstrated with type 1 and type 2 diabetes mellitus. It presents with wide range of clinical manifestations from megaloblastic anaemia pancytopenia impaired memory, dementia, delirium, peripheral neuropathy, sub-acute combined degeneration of the spinal cord,

Increased frequency of vitamin B12 deficiency among both type 1(T1DM) and type 2 DM (T2DM) patients have been documented in several cross sectional studies and case reports. The prime factor associated with vitamin B12 deficiency among patients with T2DM is Metformin use. The prevalence of vitamin B12 deficiency due to metformin use range from 5.8% to 33%. The varied study definitions of vitamin B12 deficiency explains the wide variation of the reported prevalence. Pflipsen et al. on 203 outpatient type 2 diabetic patients at a military primary care clinic in USA, definite vitamin B12 deficiency was defined as “serum vitamin B12 concentrations of <100 pg/ml or elevated serum methylmalonic acid of >243 nmol/L or homocysteine concentrations of >11.9 nmol/L if serum vitamin B12 concentrations were between 100 to 350 pg/ml”.

In the National Health and Nutrition Examination Survey of 1999–2006 in the USA “defined definite and borderline biochemical vitamin B12 deficiency as serum vitamin B12 concentrations of ≤148 pmol/l and >148-221 pmol/l respectively” .In one cross sectional study that documented a high prevalence of vitamin B12 deficiency of 33% among adult patients with T2DM vitamin B12 deficiency was defined as” serum vitamin B12 concentrations <150 pg/ml “.patients who were on high dose (>2 g/day) and longterm (4 years) metformin treatment were enrolled in this study, both clinical factors known to be associated with vitamin B12 deficiency.

Comparison of the prevalence of vitamin B12 deficiency among T2DM patients and healthy general populations is difficult, Due to the diverse definitions of vitamin B12 deficiency Used in most studies and the cultural and religious beliefs in different regions of the world.

A population based study done “among 1048 elderly Finnish subjects aged 65–100 years, the total prevalence of definite vitamin B12 deficiency was 12.1% previously diagnosed vitamin B12 deficiency was reported among 2.6% of the participants”. Vitamin B12 replacement therapy was documented among only 2.6% of the participants. In this study, vitamin B12 deficiency was defined” as total serum vitamin B12 concentrations<150 pmol/l or total serum vitamin B12 of 150–250 pmol/l and holotranscobalamin≤37 pmol/l and homocysteine≥15μmol/l.”

India, being a country with diverse cultural and religious beliefs, documented a very high prevalence of vitamin B12 deficiency among the general population due to its large proportion of vegetarians.

In one study by Yajnik et al. to determine the frequency of vitamin B12 deficiency and hyperhomocysteinemia among 441 healthy middle aged Indian men, “vitamin B12 deficiency as defined by vitamin B12 concentrations <150 pmol/L was reported among 67% of the study participants” .Vegetarian diet was the sole significant factor associated with low vitamin B12.

In another cross sectional study “among 175 healthy elderly Indian subjects aged >60 years, vitamin B12 deficiency also defined as vitamin B12 concentrations <150 pmol/L was reported among 16% of the study participants .Elevated serum MMA concentrations which are a more sensitive indicator of vitamin B12 deficiency were documented among 55% of the participants”.

Type 1 DM (T1DM) is “an auto immune condition that results from auto immune destruction of insulin secreting beta cells of the pancreas. It is invariably associated with other organ and non-organ specific auto immune and endocrine conditions leading to development of autoimmune polyglandular syndromes. Pernicious anemia resulting from chronic autoimmune gastritis is highly frequent among patients with T1DM. Chronic

autoimmune gastritis and pernicious anemia occurs in about 2% and up to 1% of the general population respectively. Among patients with T1DM, the prevalence is increased by 3 to 5 folds.

Vitamin B12 deficiency due to pernicious anemia occurs frequently among patients with T1DM. In one cross sectional study done in South India among 90 patients with T1DM, low vitamin B12 levels were noted among 45.5% of the study subjects as defined by the manufactures' cut off point of <180 pg/ml and among 54% using the published cut off point of <200 pg/ml . No positive correlation was noted between low vitamin B12 levels and gender, age, duration of DM and level of glycemic control.

Patients with T1DM actively exhibit auto antibodies to intrinsic factor (AIF) type 1 and 2 and parietal cell antibodies (PCA) especially those with glutamate decarboxylase-65 (GAD-65) antibodies and HLA-DQA1*0501-B1*0301 haplotype . The PCA inhibit secretion of intrinsic factor resulting into pernicious anemia, a condition which is 10 times more prevalent among type 1 diabetic patients than non-diabetic patients.

Type 1 AIF result into vitamin B12 deficiency by blocking the binding of vitamin B12 to IF. This prevents its transportation to its absorption site, the terminal ileum. These auto antibodies are found in 70% of patients with pernicious anemia. Primary autoimmune hypothyroidism and celiac disease are frequent co morbidities among patients with T1DM and directly

affect vitamin B12 metabolism. In one cross sectional study among 504 ambulatory T1DM patients in South Africa, the overall prevalence of co-existing auto immune hypothyroidism was 20.2%, especially among female patients (30.9% Vs 10.1%-males, $p<0.0002$).

Celiac disease in this study cohort was reported in 3 (0.6%) patients. Vitamin B12 deficiency among patients with autoimmune hypothyroidism could be explained by the presence of antibodies to the gastric parietal cells and intrinsic factor, reduced oral intake, dyserythropoesis due to thyroid hormone deficiency and defective absorption due to reduced bowel motility, bowel wall oedema and bacterial overgrowth .Celiac disease which is a highly prevalent autoimmune mediated gastrointestinal condition occurs in 1-16% of T1DM patients compared to 0.3-1% in the general population .Ingestion of wheat gluten and other related proteins have been documented to be the trigger factors of this condition in genetically susceptible individuals. Due to the associated enteropathy, patients often present with failure to thrive, chronic diarrhea and anemia due to micronutrient (mainly folate, vitamin B12) malabsorption.

Among patients with T1DM, there are no clear guidelines regarding screening for vitamin B12 deficiency. However, due to the high prevalence of pernicious anemia and subsequent vitamin B12 deficiency among T1DM patients reported in most cross sectional studies, it would be pragmatic to

screen at diagnosis and then later yearly for 3 years, then five yearly thereafter or in presence of any clinical indication since vitamin B12 deficiency can develop at any time . Screening should involve assessment of serum vitamin B12 levels and markers of gastric autoimmunity like PCA and AIF especially among T1DM patients with GAD-65 and thyroid peroxidase antibodies. Presence of these auto antibodies increases the propensity to developing vitamin B12 deficiency.

There are no guidelines to address how often patients with T1DM and T2DM should be supplemented with vitamin B12. The optimal supplementation dose of vitamin B12 is also unknown. A recently published follow up study from the United States of America showed that administration of oral vitamin B12 among type 2 DM patients on long term use of metformin was ineffective in correcting biochemical vitamin B12 deficiency .The doses of vitamin B12 in the multivitamin formulations used by the study subjects in this survey were probably inadequate to correct vitamin B12 deficiency. This stresses the need of further studies to determine the optimal vitamin B12 supplementation dose and frequency of supplementation among patients with DM. To avert vitamin B12 deficiency especially among adult type 2 diabetic patients on long term use of metformin, it is plausible to adopt a simple and cost effective supplementation approach in diabetes care. A 1000μg dose of vitamin B12

given annually would be sufficient to replenish the body's vitamin B12 stores among this category of patients.

Our study showed that the prevalence of low serum B12 in type 1 diabetics when compared with age and sex matched controls. 42 % of type 1 diabetes patients had low vitamin b 12 level using laboratory cut- off value of 180 pmol/L while only 6 % of controls had low vitamin b 12 levels(<180 pmol/l).The difference in the prevalence of low B12 levels due to different cut- off values used has been reported in many studies in the past. In addition, the lack of a gold standard complicates the diagnostic evaluations. Since serum B12 assays and other biomarkers such as MMA and holotranscobalamin lack sufficient sensitivity and specificity when used alone, a combination of markers along with clinical evaluation is preferred to define the prevalence of cobalamin deficiency. However markers such as MMA are expensive and not readily available in all laboratories. Hence serum cobalamin estimation continues to be used to assess the cobalamin status. The mean B12 value obtained in our study was very low (227.70 pmol/L), when compared with controls (308 pmol/l), which was statistically significant .however, consistent with other reports, the deficiency was similar in males and females. Correlation analysis did not show any correlation between B12 and age, duration of diabetes, or diabetic control. The presence of anti-intrinsic factor antibodies in those deficient in vitamin b 12 was done.

Among 24 type 1 diabetes mellitus patients who were deficient in vitamin b 12 (<180 pmol/l), 4 patients had anti intrinsic factor antibodies. Among 3 individuals in the control group, who were deficient in vitamin b 12 (<180 pmol/l), none of them had anti intrinsic factor antibodies, which was statistically significant “which stresses the fact that auto immune pathology for vitamin b 12 deficiency.

CONCLUSION

CONCLUSIONS

Clinical and biochemical vitamin B12 deficiency is “highly prevalent among patients with both types 1 and 2 DM. Future large and well-designed studies on screening for vitamin B12 deficiency, vitamin B12 supplementation and optimal supplementation dose among type 1 and type 2 diabetic patients are warranted to help guide formulation of guidelines in diabetes clinical care. Annual screening for vitamin B12 deficiency using more sensitive methods like serum homocysteine and methylmalonic acid concentrations (in clinical settings where they are accessible) and supplementation should be adopted among diabetic patients with specific risk factors of vitamin B12 deficiency”.

Among 24 type 1 diabetes mellitus patients who were deficient in vitamin b 12 (<180 pmol/l), 4 patients had anti-intrinsic factor antibodies. Our study demonstrated the presence of low serum B12 levels in type 1 diabetics. These findings merit further research on a larger population using additional markers to investigate into the cause of deficiency, the factors involved, and benefit of B12 supplementation in these patients.

LIMITATIONS

LIMITATIONS OF THE STUDY

- The study sample was small, and hence necessitates the need of a larger study with wide range of study populatipon.

- Other causes of vitamin b 12 deficiency should be looked for as our study detected only for anti-intrinsic factor antibodies.

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ANNEXURES

PATIENT DATA

NAME: -

AGE: - SEX: - Male / Female

OP No.:-

ADDRESS: -

CHIEF COMPLAINTS:-

Duration of Diabetes:-

Other complaints:-

DRUG HISTORY:-

PAST HISTORY:-

FAMILY HISTORY:-

PERSONAL HSTORY:-

Diet:

Habits:

Bowel & Bladder:

GENERAL PHYSICAL EXAMINATION:-

Build:	Nourishment:
Height:	Weight: BMI:
BP:	Pulse:
Pallor: P / A	Cyanosis: P / A
Clubbing: P / A	Edema: P / A
Icterus: P / A	Lymphadenopathy: P / A

SYSTEMIC EXAMINATION:-

CVS:

R/S:

CNS:

P/A:

DIAGNOSIS:

INVESTIGATIONS:

FBS:

PPBS:

HbA1c:

Vitamin b 12:

Anti-intrinsic factor antibodies:

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No. 044 25305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To
Dr.B.Midhun Kumar,
Post Graduate, MD (General Medicine),
Institute of Internal Medicine,
Madras Medical College,
Chennai – 600 003.

Dr.B.Midhun Kumar,

The Institutional Ethics Committee has considered your request and approved your study titled **"Prevalence of vitamin B12 deficiency in Type-I Diabetes Mellitus and its causes"** No.56072014.

The following members of Ethics Committee were present in the meeting held on 01.07.2014 conducted at Madras Medical College, Chennai-3.

1. Dr.C.Rajendran, M.D.,	: Chairperson
2. Dr.R.Vimala, M.D., Dean, MMC, Ch-3	: Deputy Chairperson
3. Prof.B.Kalaiselvi, M.D., Vice-Principal, MMC, Ch-3	: Member Secretary
4. Prof.R.Nandhini, M.D., Inst.of Pharmacology, MMC	: Member
5. Dr.G.Muralidharan, Director Incharge, Inst.of Surgery	: Member
6. Prof.Md.Ali, M.D., D.M., Prof & HOD of MGE, MMC	: Member
7. Prof.K.Ramadevi, Director i/c, Inst.of Biochemistry, MMC	: Member
8. Prof.Saraswathy, M.D., Director, Pathology, MMC, Ch-3	: Member
9. Prof.Tito, M.D., Director i/c, Inst.of Internal Medicine, MMC	: Member
10. Thiru S.Rameshkumar, Administrative Officer	: Lay Person
11. Thiru S.Govindasamy, B.A., B.L.,	: Lawyer
12. Tmt.Arnold Saulina, M.A., MSW.,	: Social Scientist

We approve the proposal to be conducted in its presented form.

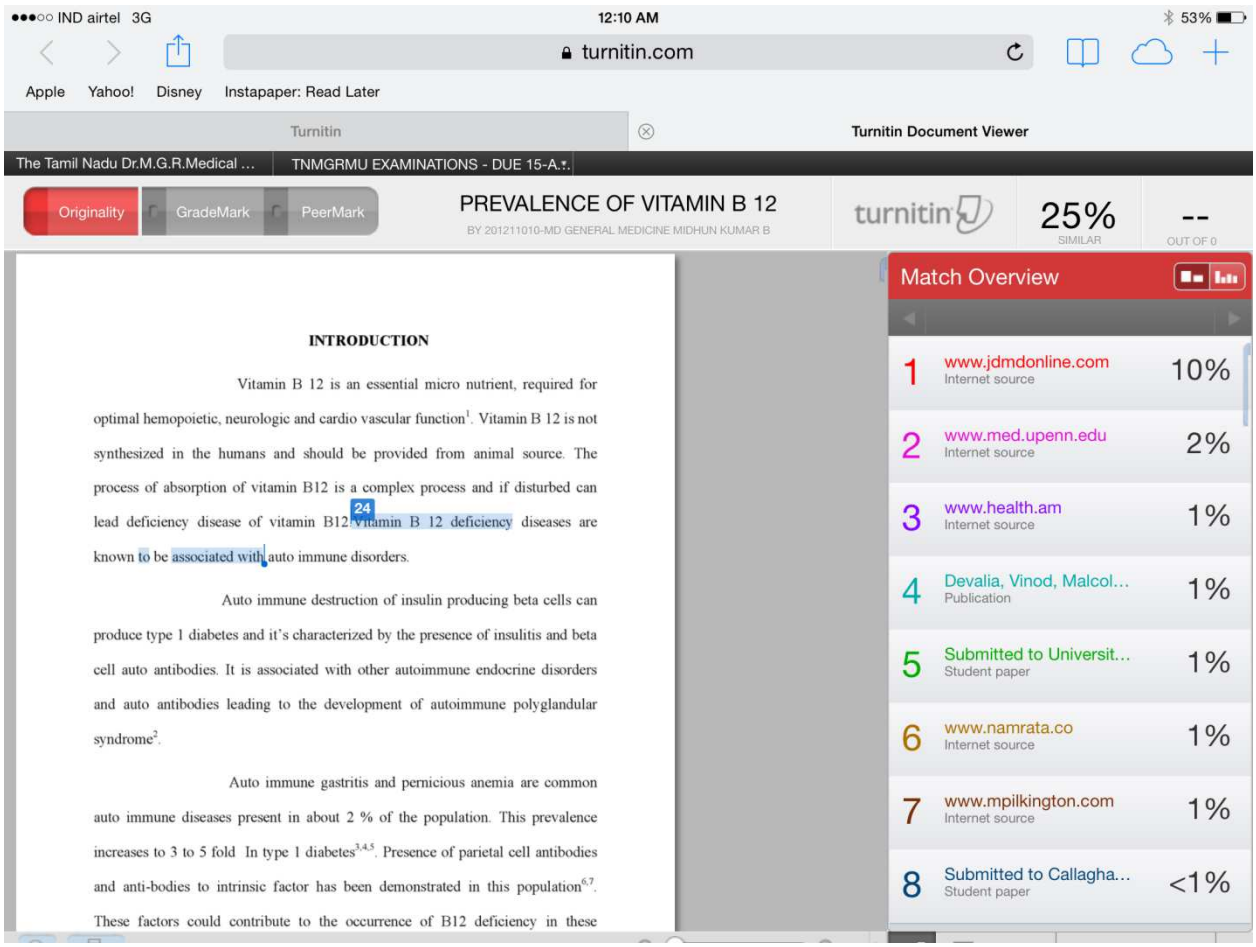
Sd/ Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

TURNITIN PLAGIARISM SCREEN SHOT



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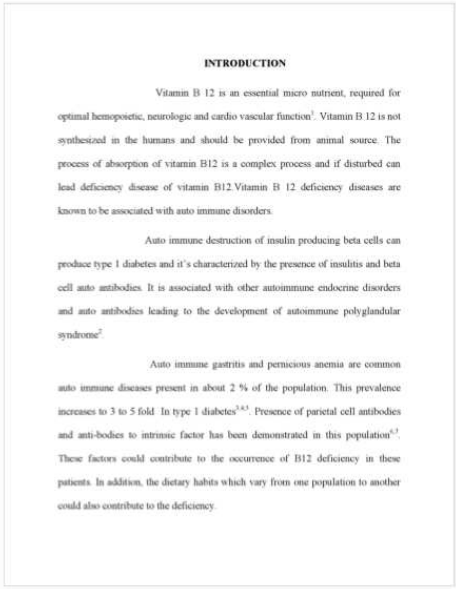

INTRODUCTION

Vitamin B 12 is an essential micro nutrient, required for optimal hemopoietic, neurologic and cardio vascular function[1]. Vitamin B 12 is not synthesized in the humans and should be provided from animal source. The process of absorption of vitamin B12 is a complex process and if disturbed can lead deficiency disease of vitamin B12.Vitamin B 12 deficiency diseases are known to be associated with auto immune disorders.

Auto immune destruction of insulin producing beta cells can produce type 1 diabetes and it's characterized by the presence of insulitis and beta cell auto antibodies. It is associated with other autoimmune endocrine disorders and auto antibodies leading to the development of autoimmune polyglandular syndrome[2].

Auto immune gastritis and pernicious anemia are common auto immune diseases present in about 2 % of the population. This prevalence increases to 3 to 5 fold In type 1 diabetes[3,4,5]. Presence of parietal cell antibodies and anti-bodies to intrinsic factor has been demonstrated in this population[6,7]. These factors could contribute to the occurrence of B12 deficiency in these patients. In addition, the dietary habits which vary from one population to another could also contribute to the deficiency.

INFORMATION SHEET

We are conducting a study on **““A STUDY ON PREVELANCE OF VITAMIN-B 12 DEFICIENCY IN TYPE 1 DIABETES MELLITUS AND ITS CAUSES”** among patients attending Rajiv Gandhi Government General Hospital, Chennai and for that your specimen may be valuable to us.

The purpose of this study is to assess the **PREVELANCE OF VITAMIN – B 12 DEFICIENCY IN TYPE 1 DIABETES MELLITUS AND ITS CAUSES.**

We are selecting certain cases and if you are found eligible, we may be using your specimen to perform extra tests and special studies which in any way do not affect your final report or management.

The privacy of the patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.

The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Signature of Investigator | Signature of Participant

PATIENT CONSENT FORM

Study Title : "A STUDY ON PREVELANCE OF VITAMIN-B 12 DEFICIENCY IN TYPE 1 DIABETES MELLITUS AND ITS CAUSES

Study Centre : Rajiv Gandhi Government General Hospital, Chennai.

Name :

Age/Sex :

Identification Number :

Patient may check (☑) these boxes

The details of the study have been provided to me in writing and explained to me in my own language ❑

I understand that my participation in the study is voluntary and that I am free to withdraw at any time without giving reason, without my legal rights being affected. ❑

I understand that sponsor of the clinical study, others working on the sponsor's behalf, the ethical committee and the regulatory authorities will not need my permission to look at my health records, both in respect of current study and any further research that may be conducted in relation to it, even if I withdraw from the study I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published, unless as required under the law. I agree not to restrict the use of any data or results that arise from this study. ❑

I agree to take part in the above study and to comply with the instructions given during the study and faithfully cooperate with the study team and to immediately inform the study staff if I suffer from any deterioration in my health or well being or any unexpected or unusual symptoms. ❑

I hereby consent to participate in this study. ❑

I hereby give permission to undergo complete clinical examination, diagnostic tests including hematological, biochemical tests and radiological tests. ❑

Signature/ Thumb impression

Signature of the investigator

Patient's name and address

Study Investigator's name

Dr. B. MIDHUN KUMAR

MASTER CHART FOR CASES

S.NO	AGE	SEX	DURATION OF DIABETES (YEARS)	FBS (mg/dl)	PPBS (mg/dl)	Hb A 1 C (%)	VITAMIN B 12 LEVEL (pg/ml)	ANTI INTRINSIC FACTOR ANTIBODIES
1	17	M	3	222	278	7.1	213	n/a
2	18	M	3	154	267	7.3	276	n/a
3	16	M	2	176	324	7.3	245	n/a
4	19	M	3	188	345	6.9	145	absent
5	18	M	3	90	145	6.4	169	absent
6	17	M	4	222	345	7.9	174	absent
7	29	M	5	101	199	7	156	absent
8	21	M	5	123	267	6.8	323	n/a
9	28	M	5	209	312	7.1	234	n/a
10	20	M	6	145	267	7.4	134	**present**
11	23	M	6	165	267	7.6	227	n/a
12	26	M	8	101	299	7.2	135	absent
13	22	M	7	167	312	6.9	324	n/a
14	21	M	4	123	178	6.4	154	absent
15	29	M	14	167	234	6.9	168	absent
16	24	M	10	227	354	7.1	170	absent
17	28	M	16	142	234	7.2	276	n/a
18	27	M	6	176	267	7.2	288	n/a
19	35	M	7	132	212	6.9	123	**present**
20	33	M	8	67	196	6.6	345	n/a
21	30	M	13	78	134	6.5	150	absent
22	31	M	12	145	238	7.1	373	n/a
23	32	M	16	110	155	7	135	**present**
24	45	M	12	122	176	7.2	178	absent
25	37	M	17	143	256	7.3	378	n/a

26	42	M	15	96	156	6.8	276	n/a
27	39	M	13	123	234	6.9	188	n/a
28	40	F	19	212	333	7	245	n/a
29	36	F	13	103	222	6.9	167	absent
30	38	F	18	98	176	6.8	175	absent
31	40	F	23	145	219	7	140	absent
32	30	F	16	78	157	6.6	233	n/a
33	35	F	19	138	186	6.9	321	n/a
34	32	F	3	174	323	6.8	268	n/a
35	33	F	12	156	267	7.2	254	n/a
36	34	F	14	198	312	7.5	167	absent
37	28	F	10	56	112	6.8	156	absent
38	27	F	4	124	178	7	267	n/a
39	23	F	5	113	189	6.9	222	n/a
40	20	F	4	87	157	6.4	236	n/a
41	24	F	3	134	178	6.8	354	n/a
42	27	F	6	189	234	7.2	164	absent
43	29	F	12	222	287	7.1	245	n/a
44	21	F	7	134	156	7.3	123	**present**
45	19	F	2	67	123	6.7	145	absent
46	17	F	1	111	167	6.8	373	n/a
47	18	F	3	154	267	7.4	345	n/a
48	19	F	1	111	167	6.9	285	n/a
49	16	F	2	156	222	6.8	267	n/a
50	42	F	19	123	267	7	276	n/a

MASTER CHART FOR CONTROLS

S.NO	AGE	SEX	vitamin b 12 levels	anti intrinsic factor antibodies
1	17	M	345	n/a
2	18	M	432	n/a
3	16	M	267	n/a
4	19	M	333	n/a
5	18	M	166	**absent**
6	17	M	221	n/a
7	29	M	278	n/a
8	21	M	345	n/a
9	28	M	367	n/a
10	20	M	234	n/a
11	23	M	432	n/a
12	26	M	234	n/a
13	22	M	456	n/a
14	21	M	378	n/a
15	29	M	345	n/a
16	24	M	367	n/a
17	28	M	333	n/a
18	27	M	176	**absent**
19	35	M	345	n/a
20	33	M	213	n/a
21	30	M	264	n/a
22	31	M	274	n/a
23	32	M	342	n/a
24	45	M	287	n/a
25	37	M	234	n/a
26	42	M	321	n/a
27	39	M	278	n/a
28	40	F	245	n/a

29	36	F	244	n/a
30	38	F	276	n/a
31	40	F	376	n/a
32	30	F	434	n/a
33	35	F	273	n/a
34	32	F	327	n/a
35	33	F	381	n/a
36	34	F	298	n/a
37	28	F	411	n/a
38	27	F	367	n/a
39	23	F	173	**absent**
40	20	F	348	n/a
41	24	F	256	n/a
42	27	F	222	n/a
43	29	F	236	n/a
44	21	F	288	n/a
45	19	F	287	n/a
46	17	F	289	n/a
47	18	F	398	n/a
48	19	F	338	n/a
49	16	F	299	n/a
50	42	F	367	n/a